12

Antibiotics

Antibiotics probably represent the greatest single contribution of drug therapy in the past half-century, a period characterized by unprecedented advancements in health care. This group of drugs provides effective control of many human microbial pathogens that previously caused prolonged incapacitation or death without appreciable regard for age, economic status, or physical fitness.

The word "antibiotic" is derived from the term antibiosis, which literally means "against life" (*anti*—against, *bios*—life). A measure of the significant and spectacular contribution of antibiotics to therapy is indicated by the common inclusion of the word in the layman's vocabulary. Most people have an accurate, or at least a functional, general concept of the word, but workers intimately involved in the antibiotic field find considerable difficulty in drafting a precise definition. The varied scientific concepts of this word reflect the viewpoints of scientific specialists, a rapidly expanding field of knowledge about all aspects of antibiotics and their applications, and such factors as a recognition of the lack of definitive separation for conditions previously considered etiologically distinct (e.g., certain neoplastic conditions and viral infections).

The most widely accepted concept defines an antibiotic as a chemical substance produced by a microorganism that has the capacity, in low concentration, to inhibit selectively or even to destroy bacteria and other microorganisms through an antimetabolic mechanism. Essentially all definitions limit antibiotics to biologic constituents that exert their action in low concentrations. This definition excludes microbial metabolites, such as ethanol, that are active against protoplasmic functions at higher concentrations. The definition of the term may be expanded by including higher plants as a source and tumors as a site of action. The concept of antibiotics as used by health-care professionals, exclusive of some individuals practicing in experimental clinics and hospitals, is limited for practical purposes to commercially available substances. Fortunately, this reduces confusion resulting from special research objectives or "antibiotics" that are too toxic for feasible therapy. A logical case can be made for including the antiplasmodial activity of quinine under an antibiotic designation, but the arbitrary exclusion of quinine from most antibiotic concepts has caused little confusion for the practitioner. The selective action of some naturally occurring compounds on the abnormal metabolism and cells of neoplasms may create greater problems in the future. Practitioners must maintain a flexible approach toward the scope of antibiotics to accommodate the applications of scientific advances.

DEVELOPMENTAL HISTORY

The history and development of antibiotics as therapeutic agents are similar to

the patterns noted for other types of drugs. Relatively ineffective attempts to use materials that are now recognized as having antibiotic associations can be detected in folk medicine and in prepenicillin scientific literature. Development in the antibiotic field since 1940 is characterized by a practical blending of empiric observation and increasingly sophisticated manipulations of biologic and chemical factors. This familiar pattern is frequently overlooked because an aura of 20th-century miracle drugs has surrounded the antibiotics.

Reports, some dating back 2500 years, indicate that various ancient and primitive peoples applied moldy bread, soybean curds, and other materials to boils and wounds liable to infection; this can be considered a folk-medicine type of antibiotic therapy. Pasteur demonstrated bacterial antagonism shortly after he established the bacterial etiology of infectious disease. During the 1880s, attempts were made to utilize antagonism to achieve an ecologic control of the human microbial flora by introducing selected nonpathogenic organisms. Pyocyanase, a crude mixture of metabolites extracted from *Pseudomonas aeruginosa*, became available around the turn of the century and could be considered the first commercial antibiotic. Pyocyanase, at best, was a poor antibiotic by modern standards, but its failure to achieve wide acceptance as a therapeutic agent can be related, in part, to the variable composition of the crude mixture and the resultant lack of reproducible or predictable therapeutic responses.

Establishment of the therapeutic feasibility of penicillin antagonism in the early 1940s stimulated the intensive efforts that have culminated in the high level of current antibiotic development. Numerous approaches to the production and use of antibiotics have been used concurrently in the past, and practical considerations of biologic, chemical, and economic factors will undoubtedly dictate a similar situation in the predictable future.

The progressive trend in the logistic aspects of antibiotic development can be illustrated by the following sequence of objectives: (1) Screen diverse sources of microorganisms for detection of useful antagonism. (2) Select improved microbial mutants, determine optimal environmental and nutritional conditions, and develop suitable procedures for recovering antibiotics from cultures. (3) Direct or induce the formation of specific, desired metabolites. (4) Modify the fermentative metabolites by biologic or chemical manipulations to yield more useful antibiotic substances. (5) Develop procedures for total synthesis of antibiotics for possible economic advantage. (6) Use an adjunct agent to modify the availability or impact of an antibiotic.

Initially, antibiotic therapy was commonly employed in a wide range of microbial infections with only limited logic or design. However, with the accumulation of experience and the availability of a greater variety of antibiotics, the trend has moved toward a more precise diagnosis of the pathologic organism, including a consideration of sensitivity variations with certain pathogens, and a more conservative use of these valuable therapeutic agents.

Production of commercial quantities of the various antibiotics involves many different approaches and procedures to accommodate the individual biologic idiosyncrasies of the producing organisms and the chemical characteristics of the individual antibiotics. A detailed consideration of antibiotic production is obviously a subject for specialized study. Fortunately, the health-science practitioner only needs a general knowledge of the production procedures and of the significance of key manipulations. This background provides a basis for understanding the scientific limits and economic components of these therapeutic agents and for comprehending readily the types of research developments that will lead to future advances and change.

SCREENING FOR ANTIBIOTICS

In searching for new antibiotics, relatively simple and rapid methods have been

developed for screening microorganisms for antibiotic-producing ability. Soil samples are commonly employed in the screen because they are a rich source of antibiotic-producing organisms (Fig. 12–1). Most of these organisms are members of a group of branching, procaryotic microorganisms that occupy a position in their morphologic characteristics between fungi and bacteria. They are placed in the taxonomic order Actinomycetales and are given the common name actinomycetes. A compilation of the microbial sources of antibiotics discovered in the United States and Japan between 1953 and 1970 reveals that approximately 85% are produced by actinomycetes, 11% by fungi, and 4% by bacteria. These facts do not detract from the significance of antibiotics from other organisms and sources, but they do suggest a greater probability for the discovery of new useful antibiotics from soil microorganisms. The antibiotics currently used in therapy are produced by surprisingly few groups of distantly related organisms. The important genera and their taxonomic relations are as follows:

Phylum Schizomycophyta
  Class Schizomycetes
    Order Eubacteriales (bacteria)
      Family Bacillaceae
        Genus *Bacillus*
      Order Actinomycetales (actinomycetes)
        Family Streptomycetaceae
          Genus *Micromonospora*
          Genus *Streptomyces*
Phylum Eumycophyta (fungi)
  Class Ascomycetes
    Order Aspergillales
      Family Aspergillaceae
        Genus *Penicillium*
  Form-Class Deuteromycetes (Fungi Imperfecti)
    Form-Order Moniliales
      Form-Family Moniliaceae
        Form-Genus *Cephalosporium*

A general method for screening first involves treating the soil sample with chemicals that inhibit the growth of interfering bacteria and fungi but do not affect actinomycetes. Cycloheximide is an antifungal antibiotic often employed for this purpose, and a 1:140 dilution of phenol is used as an antibacterial agent. Varying dilutions of the treated soil sample are streaked on agar plates containing medium that supports the growth of actinomycetes. After incubation for 3 to 7 days at 25 to 30° C, the plates are examined for characteristic colonies of actinomycetes. These colonies are

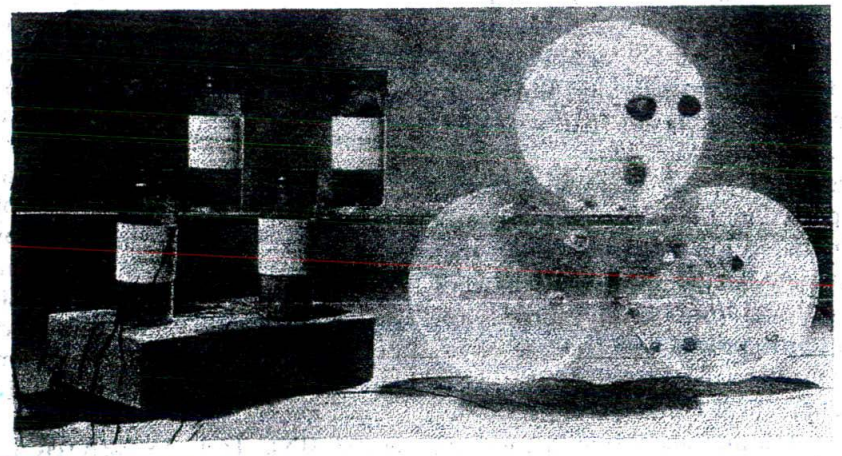


Fig. 12–1. Soil samples from various parts of the world and agar plates showing colonies of soil flora. (Photo courtesy of Eli Lilly & Co.)

then selectively transferred onto fresh medium. Giant colonies of the selected organisms are grown, and plugs are cut from the colonies that include not only the organism but also the underlying agar. If the organism produces an antibiotic, it should diffuse into the agar medium. The plugs are placed on an agar plate that has been seeded with a test organism that gives an indication of the potential usefulness of the antibiotic. For example, activity against gram-positive bacteria can be determined with *Staphylococcus aureus* or *Bacillus subtilis*, activity against gram-negative bacteria with *Escherichia coli* or *Salmonella typhi*, and antifungal activity with *Neurospora crassa*. The test plates are incubated under conditions appropriate for maximum growth of the test organism, and if after incubation there is a clear zone around the plug of the actinomycete, it can be assumed that an antibiotic in the plug inhibited the growth of the test organism (Fig. 12–2).

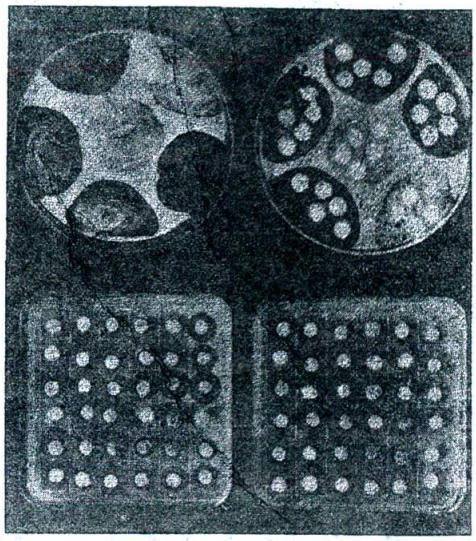


Fig. 12–2. A method for the detection of antibiotic-producing organisms: *a*, giant colonies growing on an agar plate; *b*, giant colonies with plugs removed; *c*, plugs from giant colonies showing zones of inhibition (clear zone around plug) on test plate seeded with *Staphylococcus aureus*, indicating antibiotic activity against gram-positive bacteria; *d*, plugs on test plate seeded with *Escherichia coli* showing fewer zones of inhibition, indicating little antibiotic activity against gram-negative bacilli. (Photo courtesy of Eli Lilly & Co.)

The next step in the screening procedure is to determine whether the chemical substance that produced the inhibition is a new antibiotic or a known compound. A rapid method that has been developed for this determination is termed bioautography. This assay employs paper chromatography or thin-layer chromatography and a biologic assay. An extract containing the newly discovered antibiotic is chromatographed along with reference, known antibiotics using several different solvent systems. Because each antibiotic would possess a characteristic mobility on the chromatogram in a given solvent system, a comparison of the mobilities of the unknown antibiotic with those of known antibiotics in several solvent systems would indicate whether the newly discovered antibiotic was a known compound. The detection of the antibiotics on the developed chromatogram using chemical detection methods is difficult because the antibiotics are widely diverse chemically; consequently, a biologic method is used to detect the antibiotics. By placing the developed chromatogram on an agar medium that has been seeded with an appropriate test organism, the antibiotics diffuse from the chromatogram into the agar, and after incubation, clear zones on the agar owing to inhibition of growth of the test organism indicate the position of the antibiotics on the chromatogram (Fig. 12–3).

After it is established that a microorganism has been isolated that produces a new antibiotic, quantitative assays must be employed to monitor the antibiotic titer through the various processes of production and isolation. The 2 most commonly employed assays, the turbidimetric (tube dilution) assay and the plate (agar diffusion) assay, require the use of a test organism as in bioautography. In the turbidimetric assay, the test organism is grown in test tubes that contain different concentrations of the antibiotic. There is a direct

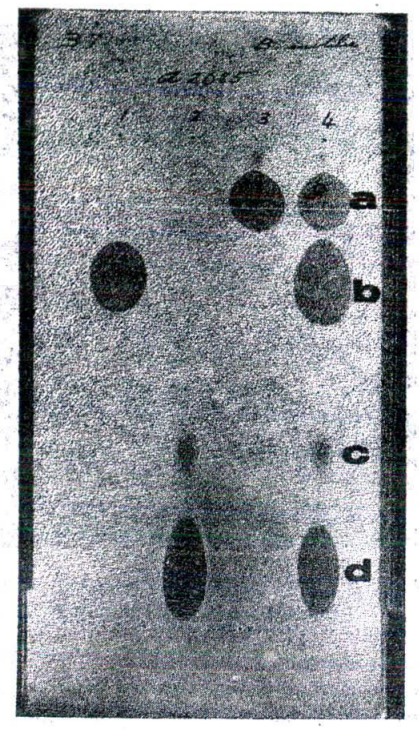


Fig. 12–3. A bioautograph with *Bacillus subtilis* as the test organism. The zones of inhibition indicate the following antibiotics were separated on the paper chromatogram: *a*, cephalexin; *b*, cephaloridine; *c*, desacetylcephalothin; *d*, cephalothin. (Photo courtesy of Eli Lilly & Co.)

relationship of the concentration of antibiotic to the growth of the test organism, and by measuring the growth of the organism, which is indicated by the turbidity of the contents of the test tube, the antibiotic titer can be determined. Clear tubes indicate a higher antibiotic concentration than turbid tubes, and the lowest concentration of antibiotic that completely prevents the appearance of turbidity is known as the **minimum inhibitory concentration** (MIC) (Fig. 12–4).

In the plate assay, filter paper discs are impregnated with solutions of antibiotic of varying concentrations, allowed to dry, placed on agar media seeded with an appropriate test organism, and incubated. As the concentration of the antibiotic increases, its diffusion through the agar medium increases; therefore, the size of the clear zone of growth inhibition around the filter paper disc is related to the concentration of antibiotic (Fig. 12–5).

COMMERCIAL PRODUCTION

When a new antibiotic has been discovered, investigations into the chemical, physical, and biologic properties of the antibiotic are required before the decision to produce the antibiotic commercially can be made. Two important requirements for production are: (1) the organism must produce the antibiotic in submerged culture as opposed to surface culture, and (2) the organism must excrete the antibiotic into the culture medium. However, some antibiotics, such as those of the polyene group, are retained in the cells of the organism and require special extraction procedures for recovery. These requirements are important considerations in production costs which, in turn, determine whether the antibiotic can compete with other antibiotics for a portion of the market. Other considerations are chemical stability, the minimum inhibitory concentration against strains of pathogenic organisms, toxic manifestations in mammals, and activity in vivo.

The commercial production of antibiotics is an excellent example of the benefits that can be achieved from a multidisciplinary approach to solving a technologic problem. One must be impressed when one thinks, on the one hand, of an obscure microorganism growing in soil and, on the other hand, of the product of that microorganism—a pure crystalline chemical substance used to save a human life. The transition from one to the other has required the most diligent application of the sciences of microbiology, chemistry, and engineering.

Commercial fermentative production of an antibiotic almost always involves growth of the producing organism in aerated tanks holding thousands of gallons of nutrient medium. Spores or occasionally vegetative growth from a stock culture of

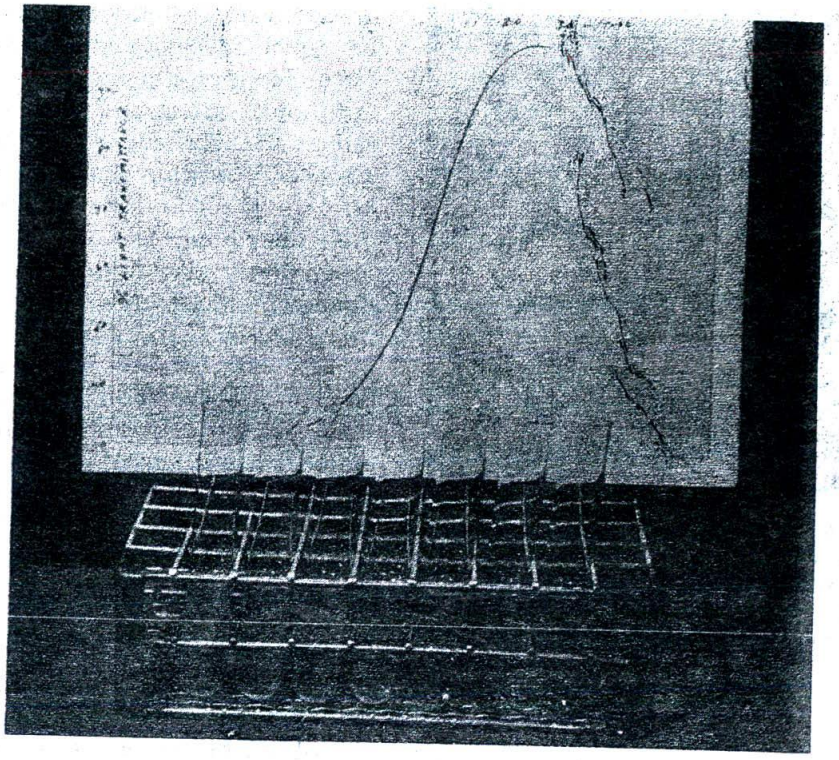


Fig. 12–4. Turbidimetric assay tubes with curve illustrating the relationship of increasing percent of light transmittance on the *y* axis and increasing concentration of antibiotic on the *x* axis. (Photo courtesy of Eli Lilly & Co.)

the organism is used to start the fermentation process. It is important to maintain stock cultures (e.g., by lyophilization) that require transfer as infrequently as possible because repeated transfer may select for those cells of the organism that are poor producers of antibiotic (Fig. 12–6). The several hundred gallons of vegetative growth that are necessary for inoculating the large fermentation tanks are obtained by successively transferring the organism to increasingly larger volumes of nutrient (Fig. 12–7). The use of a large standard inoculum reduces the incubation time required for production of the antibiotic, lessens the chance for costly contamination by foreign microorganisms, and provides the best possible opportunity for control of subtle environmental and nutritional factors that influence the antibiotic yield.

In the production of antibiotics there are often distinct phases in the fermentative process. These phases can be divided into the growth phase of the organism, which is also termed the trophophase, and the antibiotic production phase, also termed the idiophase. Figure 12–8 illustrates these phases in the course of a typical penicillin fermentation carried out in a culture medium containing glucose and lactose as the sources of carbon nutrition, corn steep liquor for nitrogen sources, and phosphate buffer. During the growth phase, the culture becomes thick owing to the formation of aggregates of fungal cells called mycelium. Growth is indicated in the figure by the curve showing an increase in mycelial nitrogen and lasts from the beginning of the culture period to approximately 1 day later (0 to 24 hours). During the growth phase, glucose rather than lactose is preferentially utilized because it can be used

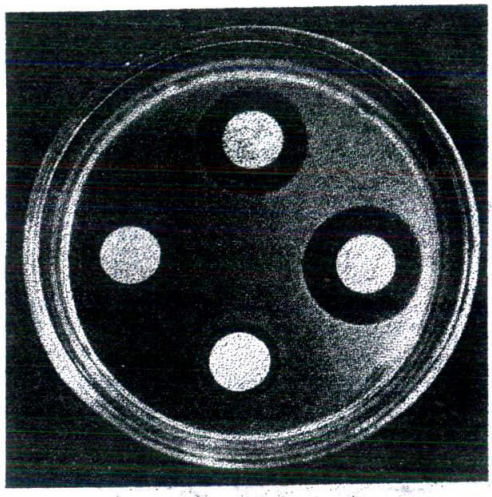


Fig. 12–5. A microbiologic assay plate showing zones of inhibition of varying size owing to different concentrations of antibiotic on the filter paper discs. The disc on the right contains the greatest concentration of antibiotic. (Photo courtesy of Eli Lilly & Co.)

directly as a source of carbon. In the growth process, ammonia is liberated by deamination of amino acids of the corn steep liquor. This liberation raises the pH of the medium to 7, the optimum pH for penicillin stability, and buffers in the medium maintain the pH close to neutrality.

Penicillin production increases rapidly between 24 to 80 hours. At the start of the antibiotic production phase, glucose has been used up, and the fungus then uses lactose for a carbon source. Little additional growth occurs because the lactose cannot be utilized until it is hydrolyzed to glucose and galactose. The decreased availability of a carbon source is thought to be the triggering mechanism for penicillin production.

INCREASING COMMERCIAL YIELD

Considerable effort is devoted to determining the optimal environmental and nutritional conditions for antibiotic production. Optimal conditions for antibiotic formation are frequently quite different from those for maximum vegetative growth. Factors that are often observed to have qualitative or quantitative importance for antibiotic production include sources of nutritional carbon and nitrogen, ratio of carbon/nitrogen nutrients, mineral composition of medium, incubation temperature, initial pH and control of pH during the fermentation period, rate and method of aeration, and addition and timing of addition of special growth- and antibiotic-promoting substances. Selection of optimal fermentation conditions is usually based on empiric observations, but careful attention to such factors is often critical. For example, some strains of *Bacillus subtilis* produce optimal yields of bacitracin when the C/N ratio is about 15; at lower ratios the yield is less, and when the ratio is reduced to approximately 6, licheniformin, a related but commercially undesired antibiotic, is produced.

The practical benefit of adding special chemicals to the fermentation cultures has probably achieved only a small fraction of its ultimate potential, but some examples will show the practical utility of this general approach. It was observed at a fairly early stage in the development of penicillin production that the addition of phenylacetamide or related compounds to the culture medium had a minor beneficial effect on the yield of penicillin substances and had a major influence on the composition of the penicillin mixture. The presence of phenylacetic acid derivatives in the nutrient mixture favored the formation of penicillin G; this reduced the problems of using a mixture of unknown or variable composition and the cost of separating the individual antibiotic substances. Use of various acyl moieties to direct the fermentative formation of other penicillins (e.g., penicillin V) achieved limited commercial success, but semisynthetic techniques have superseded this approach to the production of specialized penicillins.

The use of mercaptothiazole in cultures of *Streptomyces aureofaciens* emphasizes that additives can be beneficial without being

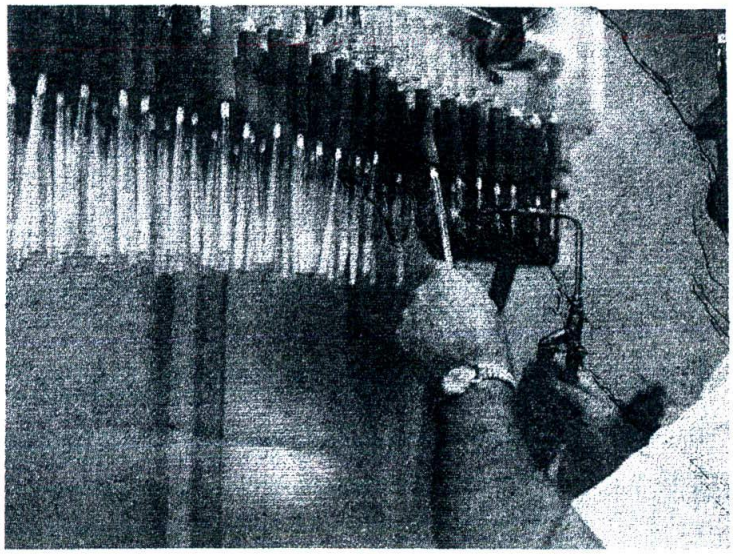


Fig. 12–6. In stock culture maintenance, the lyophilized cultures of antibiotic-producing organisms are preserved in small, sealed, glass tubes. The freeze-dried pellets in the small glass tubes will be used to start antibiotic production. (Photo courtesy of Eli Lilly & Co.)

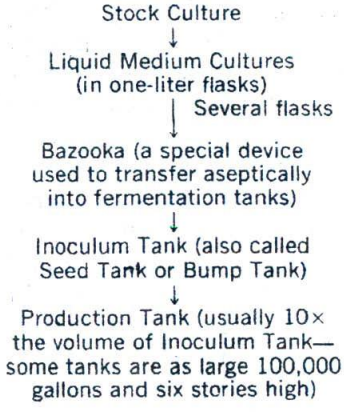


Fig. 12–7. The scale-up procedure in the commercial production of antibiotics.

incorporated into the antibiotic molecule. Strains of this actinomycete usually produce both chlortetracycline and tetracycline; the proportions depend to some degree on the availability of chloride ion in the culture medium. Tetracycline has the greater therapeutic utility, but the resolution of mixtures of these 2 tetracyclines is costly. Because the organism tends to be a chloride scavenger and because chloride ion is one of the most difficult ions to exclude quantitatively from water and nutrients, control of the presence of this ion in the nutrient medium to favor the production of tetracycline is not commercially feasible. However, the addition to the fermentation mixture of mercaptothiazole or any other compound that presumably inhibits chlorination favors tetracycline production.

Some additives may increase antibiotic production through an enzyme induction effect. For example, the addition of methionine to a cephalosporin C fermentation during the trophophase stimulates the production of the antibiotic. Because methionine does not serve as a biosynthetic precursor to the antibiotic, as compared to the role of phenylacetic acid in penicillin G biosynthesis, it is assumed that methionine stimulates the production of the cephalosporin C biosynthetic enzymes.

Conversely, it has been demonstrated that in penicillin fermentation, lysine in the culture medium inhibits antibiotic production. Penicillin and lysine are end products of a branched biosynthetic pathway in which α-aminoadipic acid is a common

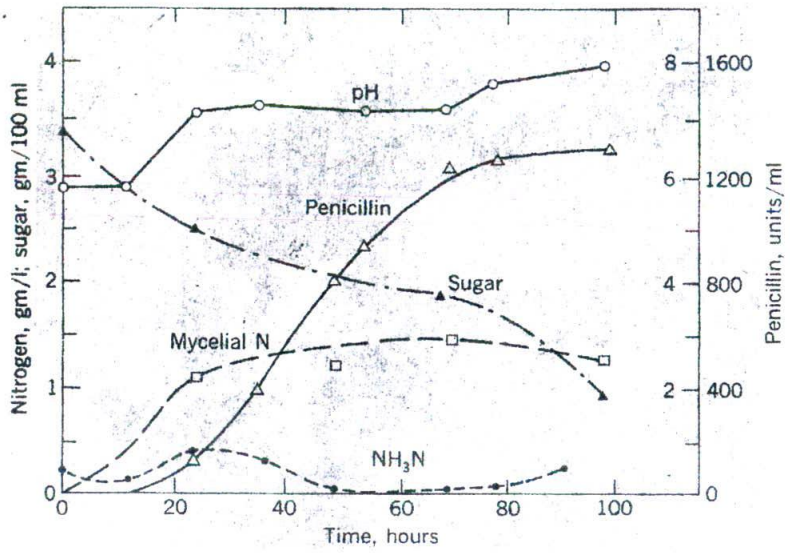
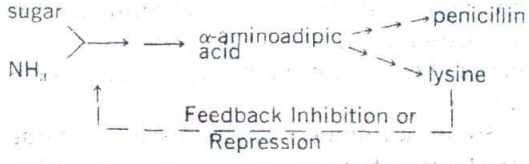


Fig. 12–8. Chemical changes, mycelial growth, and penicillin production in a typical penicillin fermentation. (Reprinted with permission from Brown and Petersen, 1950. Industrial and Engineering Chemistry, 42:1769–1774. Copyright by the American Chemical Society.)

precursor. Lysine production is regulated either by inhibition or repression of the enzymes required for the production of α-aminoadipic acid, which ultimately results in a decrease in penicillin formation.



Another important approach to increasing the yield of antibiotic is mutation and strain selection. Mutation induced by exposing the parent strain to ultraviolet light, x rays, or various mutagenic chemicals, such as nitrogen mustards and analogs of purines and pyrimidines, is the major approach for selecting improved strains, but a search of natural sources for new wild-type or different species that produce the antibiotic in higher yield than the original producing organism is also employed. In the case of induced mutations, lethal levels of the mutagen are adjusted so that approximately 90 to 99% of the cells of the organism are killed. Mutants that produce a higher yield of antibiotic are selected from the surviving cells. Penicillin production offers a good illustration of the potential success of these approaches. Penicillin antagonism was observed originally with a culture of *Penicillium notatum* Westling, which produced in surface culture 4 mg of penicillin per liter of culture medium. No mutants of *P. notatum* were found in the early selection process that would give a satisfactory yield of penicillin in submerged fermentation; however, in 1944, through natural selection, a strain of *P. chrysogenum* Thom was discovered that yielded penicillin in the amount of 40 mg per liter. Subsequently, by utilizing procedures of mutation and strain selection, the yield has been increased to 21,000 mg per liter.

RECOVERY AND ISOLATION

Most of the commercially important antibiotics are excreted readily into the nutrient medium where they accumulate. In cases such as certain of the peptide antibiotics, in which the antibiotic is retained

endocellularly until the cells reach an advanced physiologic age, the fermentation period is terminated when most of the cell membranes have undergone lysis or have lost their selective retention property. Thus, isolation of antibiotic substances is basically recovery from the culture broth. The fundamental approaches that are usually considered are selective precipitation, selective adsorption, or selective extraction with an immiscible solvent. The chemical characteristics of various antibiotics and their accompanying metabolites govern the manipulations that will be effective in any given situation. Ideally, the initial isolation procedure should be as efficient and selective as possible to give the best yield and to facilitate subsequent purification, but economic considerations commonly dictate a compromise procedure.

Precipitation is theoretically one of the best ways to recover a substance from a large volume of an aqueous mixture, but this approach has not proved commercially satisfactory for any of the therapeutically important antibiotics. The most nearly feasible application of this approach probably involves polymyxin. Polymyxin forms an insoluble helianthate complex when helianthine (methyl orange) is added to the culture broth, but this antibiotic can be recovered more economically by using an adsorption procedure. Lack of selectivity and recoverability from the precipitated complex is the most commonly cited technical disadvantage to the practical utility of this general method.

Liquid-liquid extraction using some water-immiscible organic solvent is the approach utilized for most antibiotics. This procedure lacks a high degree of selectivity with most solvents that are sufficiently inexpensive to be employed on a commercial scale. It is also relatively inefficient because antibiotic substances tend to be fairly polar molecules. However, the economic advantage of easy adaptation to a chemical engineering flow process more than offsets these limitations in most cases unless the antibiotic molecule is so polar that the partition coefficient favors the aqueous phase.

Highly polar antibiotics, such as neomycin and other aminoglycoside antibiotics, are usually recovered from the culture broth by adsorption on some suitable adsorbent. Many adsorbents remove antibiotics of this type from culture broths with varying degrees of selectivity. The major limitation to selecting adsorbents is the need to recover the antibiotics by reversing the adsorption process without using extreme conditions that would be destructive. Use of charcoal of controlled activity grades and elution of the antibiotic with dilute acid is a typical example of this isolation approach.

Once the crude antibiotic has been recovered from the nutrient broth, it is subjected to chromatography, recrystallization, or other standard manipulations to effect an appropriate degree of purification. It should be noted that attainment of chemical purity is usually considered impractical and unnecessary for therapeutic purposes. Extraneous metabolites, such as foreign proteins that cause undesirable side effects, are routinely excluded during purification, but separation of closely related antibiotic molecules is often unfeasible. Most fermentatively produced antibiotics used in therapy are actually mixtures of closely related compounds with one of the metabolites constituting the majority of the mixture. This practical approach permits reproducible therapeutic responses because a given antibiotic molecule always accounts for most of the mixture; it also provides the most economic materials for drug formulations because the inefficiency and expense of total separation of similar chemical molecules, the relative concentrations of which are unequal, can be avoided. The presence of up to 6% chlortetracycline in commercial tetracycline represents a practical application of such purification considerations. Accepted standards of purity for antibiotics and antibiotic preparations are controlled

by the *United States Pharmacopeia*. Qualitative and quantitative evaluations of antibiotic preparations for adherence to established standards utilize both biologically and chemically based tests. Colorimetric and spectrophotometric approaches and definitive measurements have largely replaced microbiologic assay and arbitrary units for quantitative purposes. However, biologic tests are still employed to detect the presence of pyrogens in parenteral antibiotic formulations. The objectives and approaches of most tests for evaluation of antibiotic preparations are not significantly different from those used to ensure the standards of other drugs.

The one unusual aspect of evaluating antibiotics is associated with the need to guarantee sterility in parenteral preparations. Masking of the presence of microbial contaminants through bacteriostatic action of the antibiotic must be precluded. Three basic approaches can be used to eliminate the antibiotic masking of microbial contaminants. Preparations containing antibiotics that are inactivated readily by biologic or chemical means may be subjected to the appropriate treatment before testing for sterility. Penicillinase inactivation of penicillin G and hydroxylamine hydrochloride inactivation of streptomycin illustrate this approach. Parenteral solutions of all antibiotics, especially those containing the more stable ones, can be evaluated by diluting the preparation such that the antibiotic level is below the minimum threshold concentration for activity or by initially removing any microorganisms with a sterile Millipore filter in a manipulation that separates the organisms from the antibiotic.

MANIPULATIVE FORMULATIONS

Effective use of many drug substances can be enhanced through various manipulations in pharmaceutic formulations. Antibiotics are no exception. Three approaches for the protection of labile antibiotic molecules, the use of insoluble derivatives to eliminate objectionable tastes and thus gain patient acceptance for certain oral formulations, and the use of either soluble or insoluble salts to facilitate the desired delivery of the therapeutic agent illustrate the practical utilization of manipulative formulations for various antibiotics.

Buffers in oral penicillin G preparations reduce the destructive effect of gastric acidity, and enteric coatings of some oral erythromycin formulations protect the macrolactone ring of this antibiotic until it passes through the acidic environment of the stomach and into the small intestine where it is absorbed. Erythromycin estolate (the dodecyl sulfate salt of the propionyl ester) and triacetyloleandomycin are much more insoluble than the parent macrolide antibiotics. This property makes a dual contribution to oral suspensions of these antibiotic substances. The insolubility helps to avoid the extremely bitter taste of these drugs and to protect them until they reach the lower intestine.

The glucoheptonate and lactobionate salts of erythromycin are used to increase the solubility of the antibiotic sufficiently to permit intravenous administration. The relatively insoluble benzathine and procaine salts of some penicillins are used intramuscularly for repository effects. When benzathine penicillin G is used in oral suspensions, this insolubility characteristic contributes a stability factor.

The use of an adjunct agent is another sophisticated approach to modifying the therapeutic availability or impact of an antibiotic. The classic example is probenecid, which inhibits the tubular excretion of penicillins. Concurrent administration of penicillins and probenecid is used to achieve prolonged blood levels of these antibiotics. Recent examples are the addition of clavulanic acid, a β-lactamase inhibitor without significant antibiotic activity per se, to a formulation of amoxicillin or ticarcillin;

the result of the combinations is an expanded therapeutic spectrum.

THERAPY AND UNDERLYING BIOLOGIC FACTORS

Various antibiotics are widely employed for the effective control of most serious infections, but prophylactic administration of antibiotics to individuals is rarely justified. Effective antibiotic therapy involves the correct diagnosis of the pathogen and the proper selection of an antibiotic. Diagnostic bacteriologic examination is usually a minimum basis for rational therapy. Exceptions include diseases such as scarlet fever, typhoid fever, or other conditions characterized by clinical symptoms that are indicative of a specific microbial etiology. Interim antibiotic therapy is usually initiated on a calculated judgment basis in acute cases of meningitis, pneumonia, urinary tract infections, and similar conditions with multiple possible causes pending bacteriologic diagnosis; the therapeutic approach is modified as necessary upon confirmation of the causative organism.

In order for the physician to exercise clinical judgment properly, he must have a knowledge of the bacteriologic statistics of infection, i.e., he must know what organisms most often produce a certain type of infection in particular areas of the body and in patients at a particular age. For example, in cases of bacterial meningitis in adults, the most common causative organisms are *Neisseria meningitidis* and *Streptococcus pneumoniae*. In children under 10 years of age, *Haemophilus influenzae* is also a common causative agent; however, in infants less than 1 month of age, coliform bacteria such as species of *Escherichia*, *Klebsiella*, and *Enterobacter* are added to the list of common causative agents (Table 12–1).

It should be emphasized, however, that rational antibiotic therapy depends first on isolating and identifying the pathogenic organism from the focus of infection and then on determining the sensitivity of that strain or organism against properly selected antibiotics known to be potentially active against the organism. Antibiotics with antibacterial activity are often classified into 2 broad categories on the basis of inhibiting predominantly gram-negative or gram-positive bacteria. Knowledge that a given antibiotic or group of antibiotics is characterized by a gram-negative or a gram-positive spectrum has some therapeutic utility, especially for selecting an antibiotic for initiating therapy in the absence of definitive bacteriologic data and for considering alternate antibiotic approaches. When the pathogen is known or strongly suspected, selection of an effective antibiotic can frequently be based on the knowledge that the spectrum of an antibiotic includes a specific microorganism. However, judicious selection of an effective antibiotic for control of *Escherichia coli* and many pathogenic species of *Klebsiella*, *Proteus*, *Pseudomonas*, and *Staphylococcus* necessitates individual determination of susceptibility because various strains of these pathogens have different antibiotic sensitivities.

Many pathogens are susceptible to more than one commercially available antibiotic. The choice of antibiotic for any given therapeutic situation must be based on composite considerations of a number of factors and is rarely unequivocal. Properties frequently cited for a clinically ideal antibiotic include a complete freedom from acute and chronic toxicities; an optimal activity near pH 7 that is not influenced by serum, other body fluids, or pus; sufficient solubility in aqueous fluids to facilitate good distribution to all body tissues; chemical stability; efficient absorption following oral administration; no tendency to induce the development of resistant strains of pathogens; and a low expense factor. No known antibiotic possesses all of these ideal characteristics. The naturally occurring penicillins probably most nearly approach many of these properties for therapeutic situations in which their spectrum

Table 12–1. A Summary of Common Pathogens That Cause Infections Treatable with Antibiotics

Pathogenic Organism	Disease Produced
Gram-positive cocci	
Staphylococcus aureus	cellulitis, impetigo, septicemia, endocarditis, meningitis, osteomyelitis, pneumonia, food poisoning, furunculosis
Streptococcus faecalis	subacute bacterial endocarditis, urinary tract infection
Streptococcus pneumoniae	pneumonia, meningitis, otitis
Streptococcus pyogenes β-hemolytic group A	scarlet fever, rheumatic fever, erysipelas, pharyngitis, impetigo
Gram-positive bacilli	
Bacillus anthracis	anthrax
Clostridium botulinum	food poisoning (botulism)
Clostridium difficile	pseudomembranous colitis
Clostridium perfringens	gas gangrene
Clostridium tetani	tetanus
Corynebacterium diphtheriae	diphtheria
Gram-negative cocci	
Neisseria gonorrhoeae	gonorrhea
Neisseria meningitidis	meningitis
Gram-negative bacilli	
Bacteroides fragilis	abscesses of abdomen, lung, brain
Bordetella pertussis	whooping cough
Brucella abortus, B. melitensis, and *B. suis*	brucellosis
Enterobacter aerogenes	pneumonia, wound infections, urinary tract infection
Escherichia coli	urinary tract infection, septicemia, respiratory infections, peritonitis
Haemophilus influenzae	respiratory infections, meningitis, otitis
Klebsiella pneumoniae	pneumonia, urinary tract infection, septicemia
Legionella pneumophila	Legionnaire's disease
Proteus vulgaris	urinary tract infection, septicemia
Pseudomonas aeruginosa	urinary tract infection, pneumonia, burn-wound infection, septicemia
Salmonella species	food poisoning (salmonellosis)
Salmonella typhi	typhoid fever
Shigella dysenteriae	bacillary dysentery
Vibrio cholerae	Asiatic dysentery
Yersinia pestis	bubonic plague
Acid-fast bacilli	
Mycobacterium leprae	leprosy
Mycobacterium tuberculosis	tuberculosis
Spirochetes	
Treponema pallidum	syphilis
Fungi	
Blastomyces dermatitidis	North American blastomycosis
Candida albicans	candidiasis (moniliasis)
Coccidioides immitis	coccidioidomycosis (San Joaquin fever)
Cryptococcus neoformans	cryptococcosis
Histoplasma capsulatum	histoplasmosis
Epidermophyton, Microsporum, and *Trichophyton* (various species)	dermatomycoses (ringworm, athlete's foot)
Miscellaneous, Rickettsiae, Large Viruses	
Mycoplasma pneumoniae	respiratory infections
Rickettsia prowazekii	epidemic typhus
Rickettsia rickettsii	Rocky Mountain spotted fever
Rickettsia typhi	endemic typhus
Chlamydia psittaci	psittacosis (parrot fever)
Chlamydia trachomatis	trachoma, postgonococcal urethritis

is adequate because they tend to have a rapid onset of systemic activity when orally administered, cause a low incidence of toxicity, and are inexpensive. However, serious penicillin hypersensitivities contraindicate the use of these antibiotics in some individuals, and the development of resistance by some pathogens is a definite therapeutic concern. Cost is never a major or exclusive criterion for selection of a first-choice antibiotic for therapeutic purposes, but if all other factors are equal, the least expensive therapeutic approach (not necessarily the least expensive unit formulation) serves the best interests of the patient.

Properties of the antibiotic per se are not the only considerations in selecting the best therapeutic agent. Such factors as age and secondary debilitating conditions may influence the use or choice of antibiotics in specific situations. The following examples illustrate generally the situations that may be encountered. Gradual development of normal renal function during the neonatal period necessitates adjustment in the dosage and administration interval when employing antibiotics that are eliminated by the kidneys. An antibiotic that is excreted in the urine also must be used cautiously for systemic purposes in adult patients with renal complications, and chloramphenicol is usually considered an antibiotic of last resort when an infection is accompanied by hematopoietic abnormalities. When serious gastrointestinal complications would contribute to erratic absorption upon oral administration, the parenteral features of an antibiotic become dominant.

Data are being accumulated on the modes and mechanisms of action of various antibiotics, on the bases for toxicities in antibiotic therapy, and on the details of resistance. The available information is sufficient to rationalize scientifically many developments that may be observed during therapy. A consideration of these factors will undoubtedly provide a basis for more effective and precise antibiotic therapy in the future when more complete knowledge becomes available.

MODES AND MECHANISMS OF ACTION

A number of different classification schemes could be used to categorize the selective toxicity of antibiotics for susceptible microorganisms. The recognition of 4 general modes of action, namely, inhibition of microbial cell-wall formation or biosynthesis of some essential protein, disruption of deoxyribonucleic acid metabolism, and alteration of normal function of the cellular membrane, is satisfactory pending the accumulation of more data. It is frequently difficult to distinguish primary from referred responses in preliminary attempts to determine the mode of antibiotic action. When more detailed information becomes available, current concepts on the mode of action of a few antibiotics (Table 12–2) may be altered, and the relative therapeutic importance of alternate modes of action will be clarified for antibiotics that give experimental indications for more than one general basis for their antagonistic effects.

The mechanism of action of an antibiotic, as contrasted with the general mode of action, is frequently an individualistic feature, and distinctive mechanisms of action are often observed for 2 antibiotics with the same mode of action. Precise knowledge of the mechanism of action offers tremendous potential for sophisticated developments in antibiotic therapy. Sufficient information is available on the mechanism of action of certain antibiotics that interfere with cell-wall formation and protein biosynthesis to show representative patterns of biologic involvement.

Inhibition of cell-wall formation involves the disruption of mucopeptide synthesis. Gram-positive bacteria are particularly susceptible to antibiotics that inhibit mucopeptide formation because they possess a cell wall that contains a relatively thick mu-

Table 12–2. General Mode of Antibiotic Action

Inhibition of cell wall formation	Inhibition of protein biosynthesis
Bacitracin	Amikacin
Cephalosporins	Chloramphenicol
Cycloserine	Clindamycin
Penicillins	Erythromycin
Vancomycin	Gentamicin
Other β-lactam antibiotics	Kanamycin
	Lincomycin
	Neomycin
Disruption of deoxyribonucleic acid metabolism	Netilmicin
	Paromomycin
	Spectinomycin
	Streptomycin
Actinomycin D	Tetracyclines
Doxorubicin	Tobramycin
Mitomycin C	Troleandomycin
Novobiocin	
Plicamycin	
Rifampin	Alteration in cellular membrane function
Bleomycin	
	Amphotericin B
	Colistin
	Nystatin
	Polymyxin

copeptide layer to provide structural support of the cytoplasm. The mucopeptide layer is known variously as murein, glycopeptide, or peptidoglycan, and because of the nature of its chemical structure, it is tough and fibrous. Support is required because gram-positive bacteria concentrate low-molecular-weight metabolites such as amino acids and nucleotides, which impart a high internal osmotic pressure. On the other hand, gram-negative bacteria have a relatively low internal osmotic pressure with a thin layer of mucopeptide.

The synthesis of mucopeptide occurs in distinct steps. The first step is a series of reactions inside the cell that result in the production of the basic building units (uridine diphospho-*N*-acetyl-muramylpentapeptide). Cycloserine inhibits the formation of the pentapeptide portion of the building block. In the next step, the building units are carried to the outside of the cell membrane. During this process, the units are linked covalently to the preexisting cell wall. Vancomycin and bacitracin inhibit this step of the biosynthesis. The final stage of the biosynthesis is the cross-linking of linear molecules to form the highly cross-linked, 3-dimensional mucopeptide. The last reaction in mucopeptide formation is catalyzed by a transpeptidase that splits the terminal D-alanine residues of the pentapeptide of the building unit and, in the case of *Staphylococcus aureus*, forms a peptide bond between the terminal glycine of a pentaglycine bridge and the penultimate D-alanine of a mucopeptide strand (Fig. 12–9). Therefore, each polypeptide side chain of each repeating building unit becomes covalently linked to the side chains of neighboring mucopeptide strands. The cross-linking process has 2 steps: carboxypeptidation, followed by transpeptidation (Fig. 12–9). The penicillins and the cephalosporins are competitive inhibitors of this cross-linking.

Although the precise mechanism is not known, it appears that the penicillin or cephalosporin molecule occupies the D-alanyl-D-alanine substrate site of the DD-carboxypeptidase and/or the peptidoglycan transpeptidase, forming a covalent adduct that is stable to subsequent hydrolysis and, therefore, irreversibly inactivates the enzyme. These penicillin-sensitive enzymes are also known as penicillin-binding proteins (PBPs). The PBPs are found in the cell membrane of all bacteria examined to date. Bacterial membranes yield multiple PBPs ranging in number from 3 in gonococci to 10 or more in *Escherichia coli*. On sodium dodecylsulfate-polyacrylamide gels, PBPs have molecular weights usually ranging from 40,000 to 120,000 and are numbered in order of decreasing molecular weight. The currently used nomenclature involves assigning numbers to the protein, with 1 being the highest molecular weight; therefore, the numerical connotation of PBPs is strictly a reference to their relative molecular size within the group of PBPs detected in a microorganism. Thus, PBP-1 of *Esch-*

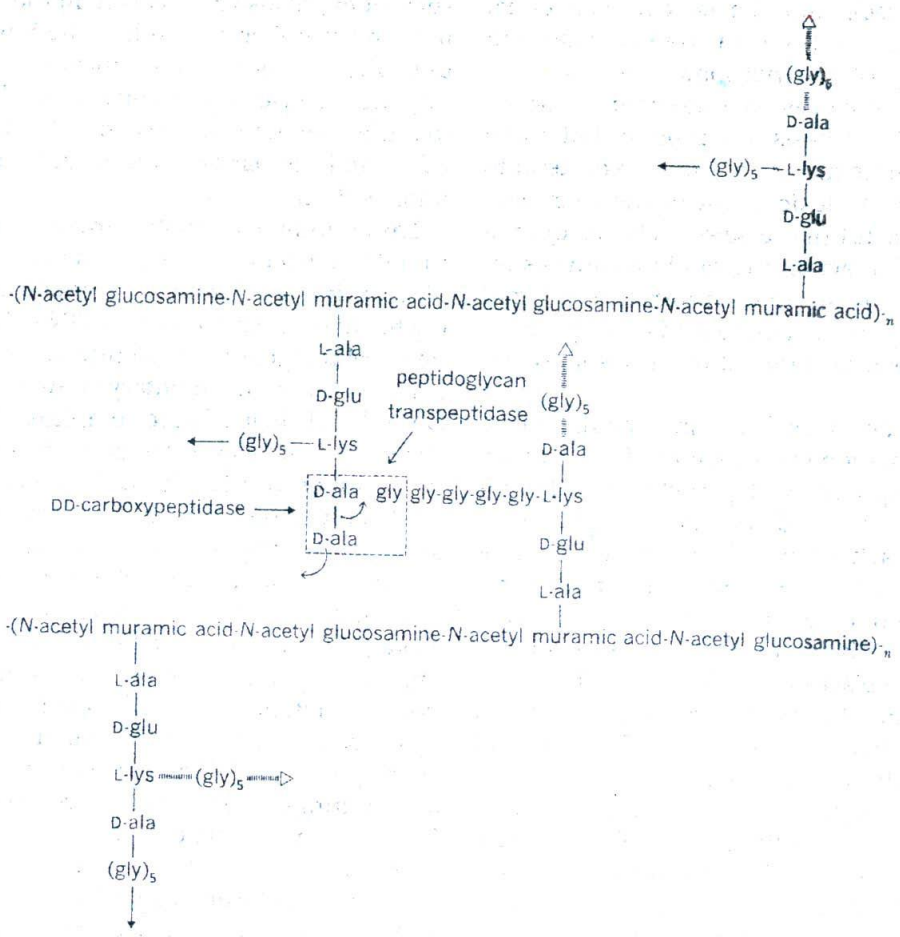


Fig. 12–9. Partial structure of bacterial cell wall mucopeptide showing polysaccharides composed of polymeric chains of alternating units of *N*-acetylglucosamine and *N*-acetylmuramic acid. The peptide chains cross-link the polysaccharide chains to make a rigid 3-dimensional structure. The last steps in mucopeptide formation are catalyzed by a DD-carboxypeptidase that splits off the terminal D-alanine and peptidoglycan transpeptidase that forms a peptide bond between the penultimate D-alanine and, in the case of *Staphylococcus aureus*, the glycine of a pentaglycine bridge.

erichia coli need not have anything in common with PBP-1 of gonococci.

The physiologic function of PBPs in the formation of cell wall has been assigned so far in only a limited number of bacteria. In *Escherichia coli*, PBP-1, PBP-2, and PBP-3 all have transpeptidase activity and are involved, respectively, in elongation, shape, and septation during growth. β-Lactam antibiotics that bind to PBP-1 cause cell lysis, whereas binding to PBP-2 produces giant spherical shaped cells; those binding to PBP-3 produce filamentation. Most β-lactam antibiotics bind to PBP-1 and PBP-3, whereas amdinocillin binds preferentially to PBP-2.

Formation of an essential protein may be blocked at any of the basic stages of protein biosynthesis. The antibiotic could adversely influence the replication and synthesis of DNA, the transcription of the genetic code and the specific sequential

synthesis of DNA, the transcription of the genetic code and the specific sequential synthesis of mRNA, or the synthesis and assembly of the ribosomes. All of these biologic processes are fundamental for the eventual synthesis of a protein, but many constituents that act at these levels tend to be relatively toxic. Most of the therapeutically useful (more selectively toxic) antibiotics that act on protein biosynthesis influence in some manner the normal assembly of the amino acids into proteins at the surface of the mRNA-ribosome complex.

The ribosomes found in bacteria have a sedimentation coefficient of 70S, and they are composed of 2 particles of different size, the 50S and the 30S ribosomal subunits. Each subunit is composed of ribosomal RNA and a number of different proteins. Antibiotic action to inhibit protein biosynthesis can be focused on the events that take place on the ribosomes. These are initiation, binding of aminoacyl-tRNA, peptide bond formation, translocation, and termination (Fig. 12–10).

Streptomycin, an aminoglycoside antibiotic, affects initiation as well as elongation and termination of protein synthesis. The antibiotic binds to the 30S subunit and causes a breakdown of the initiation complex, resulting in the release of f-met-tRNA. Other aminoglycoside antibiotics such as kanamycin, gentamicin, and neomycin interfere with initiation of protein synthesis; however, these antibiotics affect elongation of the peptide chain more markedly through inhibition of translocation than through initiation. In addition, the aminoglycoside antibiotics with a streptamine or 2-deoxystreptamine moiety provoke codon misreading or induce the uptake of incorrect amino acids that do not correspond to the codon. The tetracyclines interfere with the binding of aminoacyl-tRNA to the acceptor site of the 70S ribosome. Experimental evidence points to a single strong binding site for tetracycline located on the 30S subunit; however, it has not been completely ruled out that the 50S subunit might also be involved in the binding. Chloramphenicol binds to the 50S subunit where it disrupts the function of peptidyl transferase. Erythromycin also binds to the 50S subunit. It does not inhibit peptide bond formation, but it does block translocation.

The antitumor antibiotics and others disrupt DNA metabolism. Actinomycin D and plicamycin bind through hydrogen bonding to guanine residues of the DNA double helix. Mitomycin C covalently cross-links between the complementary strands of the DNA double helix. These complexes of the drug with the DNA template block the transcription of RNA by DNA-dependent RNA polymerase which, in turn, is responsible for the antitumor effect.

Other antibiotics affect the permeability of the cell membrane in a way that causes leakage of cytoplasmic solutes. The 2 most important groups of these drugs are the polyene antibiotics, amphotericin B and nystatin, and the peptide antibiotics, such as the polymyxins. The polyene antibiotics are antifungal agents that affect the membranes of eucaryotic cells but have no activity on bacteria. This difference in sensitivity of different organisms to these antibiotics is determined by the presence of sterols in the cell membrane of eucaryotic cells. The polyenes bind to the membrane and the extent of binding is proportional to the amount of sterol present. Molecular models show the polyenes to have a rodlike structure held rigid by an all-*trans* extended conjugated system that is equal in length to a sterol molecule. One surface is lipophilic and the opposite, studded with hydroxyl groups, has a hydrophilic face (see page 366). There is evidence that there is a packing of alternating sterol and polyene molecules, which creates a pore through the cell membrane. The pore is thought to be a hollow cylinder with a polar interior surface caused by the hydrophilic hydroxyl groups of the polyene molecules and an exterior surface com-

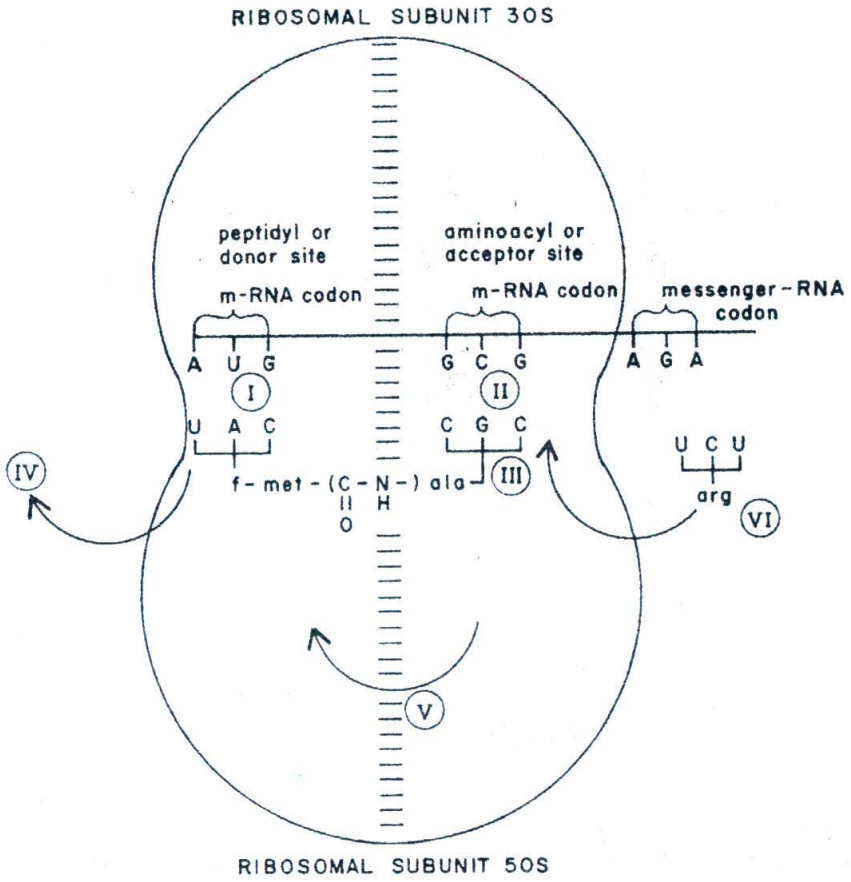


Fig. 12–10. Sequence of events of protein synthesis on the 70S ribosome:

I. Formation of initiation complex. Involves the binding of the first aminoacyl-tRNA to the ribosome. In bacteria, the first amino acid bound is formylmethionine (f-met).
II. Binding of the next aminoacyl-tRNA to aminoacyl site.
III. Formation of peptide bond catalyzed by a ribosome-bound peptidyl transferase.
IV. Release of formylmethionine-specific tRNA.
V. The peptidyl-tRNA (f-met-ala-tRNA) moves to the peptidyl site. This is called the translocation step, and the mRNA shifts to the next codon.
VI. The aminoacyl site is free and available for the next addition of aminoacyl-tRNA which, in this case, is arginine-tRNA.

posed of sterols being attracted to the lipophilic side of the polyenes (Fig. 12–11). Ions from the cytoplasm such as K^+ would leak through the polar pore, causing damage to the cell by the upset of the ion balance. The peptide antibiotics also bind to the cell membrane and disturb membrane function; however, these antibiotics are active against bacteria since sterols are not required for binding.

BASES OF TOXICITY

One limitation to the therapeutic use of an antibiotic substance is mammalian toxicity. The manifestation of such adverse reactions varies greatly with different antibiotic molecules. Basically, these side effects are an extension to mammalian biologic processes of the mechanisms of antibiotic action, hypersensitivity, or a

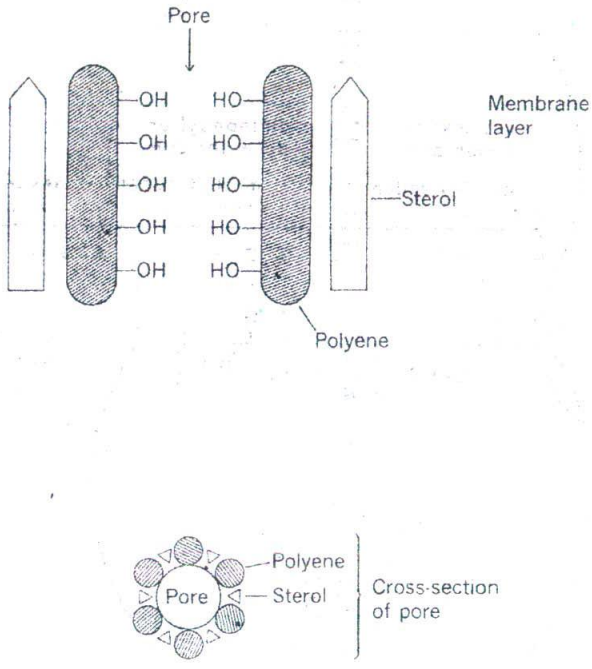


Fig. 12–11. The possible interaction of polyene antibiotics in a eucaryotic cell membrane. The polyenes complex with membrane sterols to form a pore through the membrane through which ions may pass.

pharmacologic action that is independent of the antibiotic activity of the molecule. An antibiotic that acts by inhibiting protein synthesis in susceptible microorganisms is potentially toxic to mammalian systems involving the same or related essential proteins. Theoretically, the safest antibiotic inhibits an essential process, such as cell-wall formation, that is unique to the microorganism. Actual situations usually follow the theoretical considerations, as illustrated by the relative safety of the penicillins and the relative toxicity of chloramphenicol. However, some degree of deviation from the ideal is probably universal because antibiotic molecules normally lack absolute specificity or the ability to influence only one biochemical reaction. In the case of penicillins, hypersensitization with serious consequences precludes the use of these antibiotics in some individuals. Many antibiotic molecules are characterized by reactive functional groups, and hypersensitivity may be a problem with molecules containing functional oxygen groups that can react with proteins to yield a potentially antigenic hapten-protein molecule. Toxicity caused by some independent pharmacologic property of the antibiotic is usually difficult to predict; this type of complication must be evaluated individually for each antibiotic.

In addition to any adverse pharmacologic action of an antibiotic per se, indirect toxicities can be observed with these therapeutic agents. The most common type of indirect antibiotic-induced toxicity is associated with an alteration in the ecologic balance of the intestinal flora. This problem is greatest with the broad-spectrum antibiotics because a major portion of the intestinal flora may be suppressed. *Candida*

albicans is an example of the slow-growing, unsusceptible microorganism that may become a dominant component of the intestinal flora following the administration of antibiotics. The body frequently has no prior adaptation or tolerance to the level of foreign metabolites resulting from the unusual proliferation of such organisms; the toxicity is usually manifest as gastrointestinal disturbances rather than as acute toxicities. The pseudomembranous colitis caused by *Clostridium difficile* in some patients receiving clindamycin is another example of such clinical problems.

MODES OF RESISTANCE

Antibiotic resistance is a major therapeutic concern. One practical way to circumvent this problem, at least for short-term purposes, is to develop and use new antibiotics, but experts are concerned justifiably about the practicality of long-term developmental aspects of this approach. Resistance to antibiotics may result through spontaneous or induced genetic mutation. However, many of the practical problems have developed via the process of selection or, in other words, favoring through the use of antibiotics the low frequency of organisms of antibiotic-resistant genotype that exists naturally in the antibiotic-sensitive, wild population. Spontaneous mutation is believed to make only a minor contribution to the total problem of antibiotic resistance. Bacterial cells can acquire genetic material from other bacterial cells through the processes of transformation, transduction, and conjugation. Transformation, which is a process by which DNA from a lysed bacterial cell is inserted directly into a recipient cell, makes no substantial contribution to the clinical problem of drug resistance. Transduction, or the phage-induced transfer of resistant determinant sections of bacterial DNA, is believed to be an important factor in the emergence of drug-resistant strains of *Staphylococcus*. Conjugation is a widely recognized mechanism for transmitting resistance among gram-negative bacilli of clinical concern. Conjugation of compatible cells (which may represent different species or even genera) provides a means for direct transfer of R-factor genes residing on bacterial episomes, and great danger lies in the fact that bacterial episomes may contain genetic information for multiple resistance.

Multiple mechanisms of resistance to many antibiotics appear to exist, and the lack of precise information in many cases makes general categorization difficult. However, some modes of resistance that can be noted include:

1. Enzymatic inactivation of the anti-
2. Altered permeability of the pathogen to the antibiotic;
3. Development of altered, less sensitive enzymes or of alternate metabolic pathways in the pathogen.

The β-lactamase inactivation of penicillins and cephalosporins is by far the best documented mechanism leading to antibiotic resistance. The significance of penicillinase was recognized at an early date in antibiotic therapy, and the semisynthetic penicillins are a direct result of efforts to avoid the specificity of the enzyme. A penicillin amidase also occurs in some microorganisms; this amidase yields the inactive 6-aminopenicillanic acid, but this type of penicillin inactivation does not appear to contribute significantly as a means of pathogenic resistance in any actual therapeutic problem.

Gram-negative bacteria bearing any of several R-factors for multiple resistance can enzymatically inactivate aminoglycoside antibiotics by forming either phosphoryl, adenyl, or acetyl derivatives of these antibiotics.

Altered permeability is a frequently mentioned mode of resistance. Actual substantiation of this type of involvement is limited. Tetracycline resistance in *Escherichia coli* appears to be related to a decrease in the ability of the bacterial cells to take

up the antibiotic. Another possible example is chloramphenicol. One biochemical basis of resistance to this antibiotic is an acquired selective impermeability of cellular membranes of some organisms; however, it is also known that certain strains of *E. coli* that are resistant to chloramphenicol enzymatically inactivate the antibiotic by acetylation.

Resistance caused by the development of altered enzymes or metabolic pathways is poorly documented in the current scientific literature. This general mode of resistance is recognized, e.g., certain resistant strains of *Bacillus subtilis* that fail to bind erythromycin at the 50S subunits on the ribosomes, but the overall therapeutic significance of this type of resistance is relatively unknown.

ANTIBIOTICS DERIVED FROM AMINO ACID METABOLISM

The commercially available and therapeutically useful antibiotics can be classified on the basis of the biosynthetic origin of the antibiotic molecules. These useful microbial metabolites are products of amino acid, acetate, and carbohydrate metabolism. Only one of the basic groups of metabolites is involved in the formation of most medicinally important antibiotics, but in the case of some, such as the macrolides, precursors from diverse metabolic origins are combined to yield the antibiotic molecule.

Antibiotics derived from amino acids include the penicillins, the cephalosporins, chloramphenicol, cycloserine, dactinomycin, and the polypeptide antibiotics (e.g., bacitracin, polymyxin). Considerations of chronology, sophisticated state of current development, and significance suggest the penicillins for initial monographic coverage.

Penicillins

Penicillin antagonism attracted the attention of Sir Alexander Fleming in 1928. Fleming was studying staphylococci at St. Mary's College in London when he noticed a zone of inhibition surrounding a *Penicillium* contaminant in one of his cultures. The *Penicillium* was initially identified as *P. rubrum* but was later determined to be *P. notatum*. Interest in this antagonism remained largely academic until after 1940. In 1938, Florey and Chain at Oxford University first isolated a crude penicillin mixture from the mold, and during the early 1940s, the therapeutic potential of penicillin was demonstrated.

Conditions in England during the first half of World War II were such that efforts to determine suitable procedures for producing commercial quantities of the antibiotic were conducted primarily in the United States. Significant early discoveries included the influence of nutrient composition on penicillin production and the discovery of a strain of *P. chrysogenum* that would produce the antibiotic in submerged fermentation. The presence of phenylpropanoid or phenylacetyl derivatives in the nutrient medium favored the formation of benzylpenicillin (penicillin G). Other penicillins could be formed by adding the appropriate precursor moieties to the fermentation cultures; penicillin V is an example of a therapeutically useful penicillin that was prepared initially by this type of manipulated biologic process (Fig. 12–12).

Discovery in the late 1950s of a strain of *P. chrysogenum* that accumulated high yields of 6-aminopenicillanic acid provided an alternate approach to preparing unusual penicillins, such as penicillin V, and provided an opportunity for even greater modification in the antibiotic molecules. 6-Aminopenicillanic acid has no significant antibiotic activity per se, but this biologically prepared substance can be chemically acylated to give a wide variety of active molecules. Amdinocillin, ampicillin, amoxicillin, azlocillin, bacampicillin, carbenicillin, cloxacillin, cyclacillin, dicloxacillin, methicillin, mezlocillin, nafcillin,

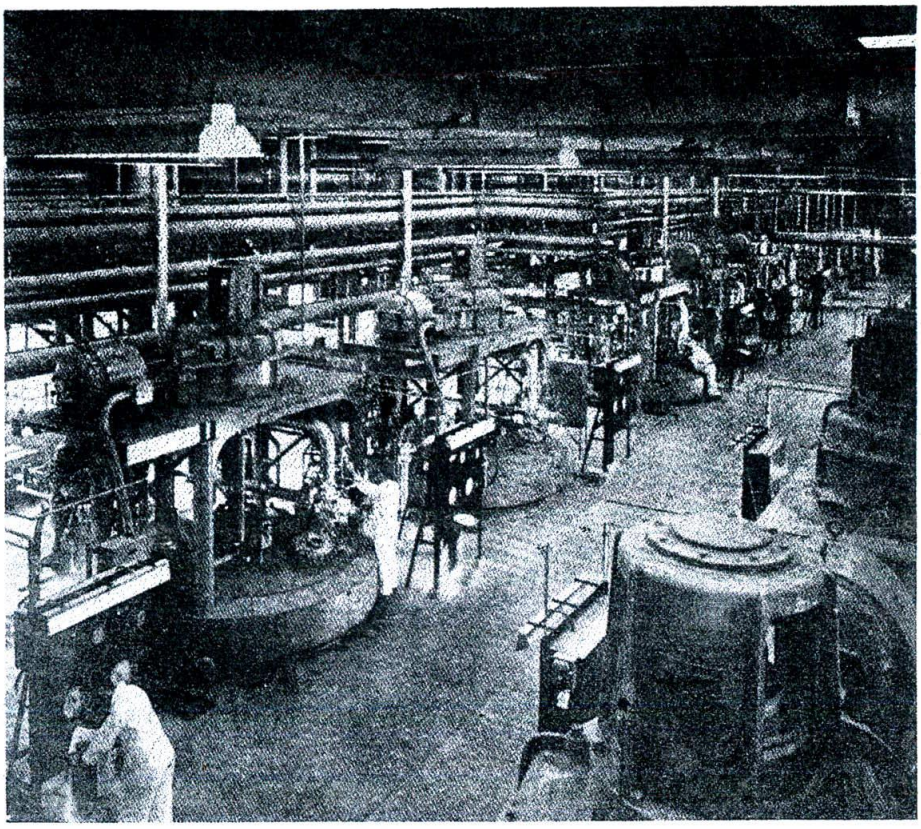


Fig. 12–12. Antibiotic fermentation area. Final fermentation takes place in these 18,000-gallon tanks. The tanks are 30 feet high but are buried to within a few feet of their tops. A maze of pipes carries water, steam, and air into the area. Storage tanks of 30,000-gallon capacity are located nearby. (Photo courtesy of Eli Lilly & Co.)

oxacillin, piperacillin, and ticarcillin are therapeutically utilized semisynthetic penicillins that have been selected for various advantages offered by their chemical, physical, or spectral properties. Structures of the commercially available penicillins are shown in Figure 12–13.

BIOSYNTHESIS OF PENICILLINS. The amino acids cysteine and valine are incorporated into the 6-aminopenicillanic acid portion of penicillin molecules, and the acyl group of penicillin G is derived from phenylacetic acid. Many of the details of the biosynthetic pathway require further clarification. It is generally believed that terminal steps in the pathway involve introduction of the characteristic acyl group and the action of an acyl transferase on isopenicillin N is suspected (Fig. 12–14). The tripeptide, δ-(α-aminoadipyl)-cysteinylvaline, is the presumed precursor of isopenicillin N, and dehydrogenation involving the mercapto function and one of the methyl groups of the valine residue of some metabolite of this tripeptide appears to yield the nucleus of cephalosporin C.

PROPERTIES OF THE PENICILLINS. The chemical structure of the penicillin nucleus is unusual and is characterized by a 4-membered β-lactam ring fused to a thiazolidine ring. This ring system contains 3 asymmetric carbon atoms in a fixed spatial arrangement, and any disruption of this arrangement by rupturing either the β-lactam ring or the thiazolidine ring results in a complete loss of antimicrobial activity.

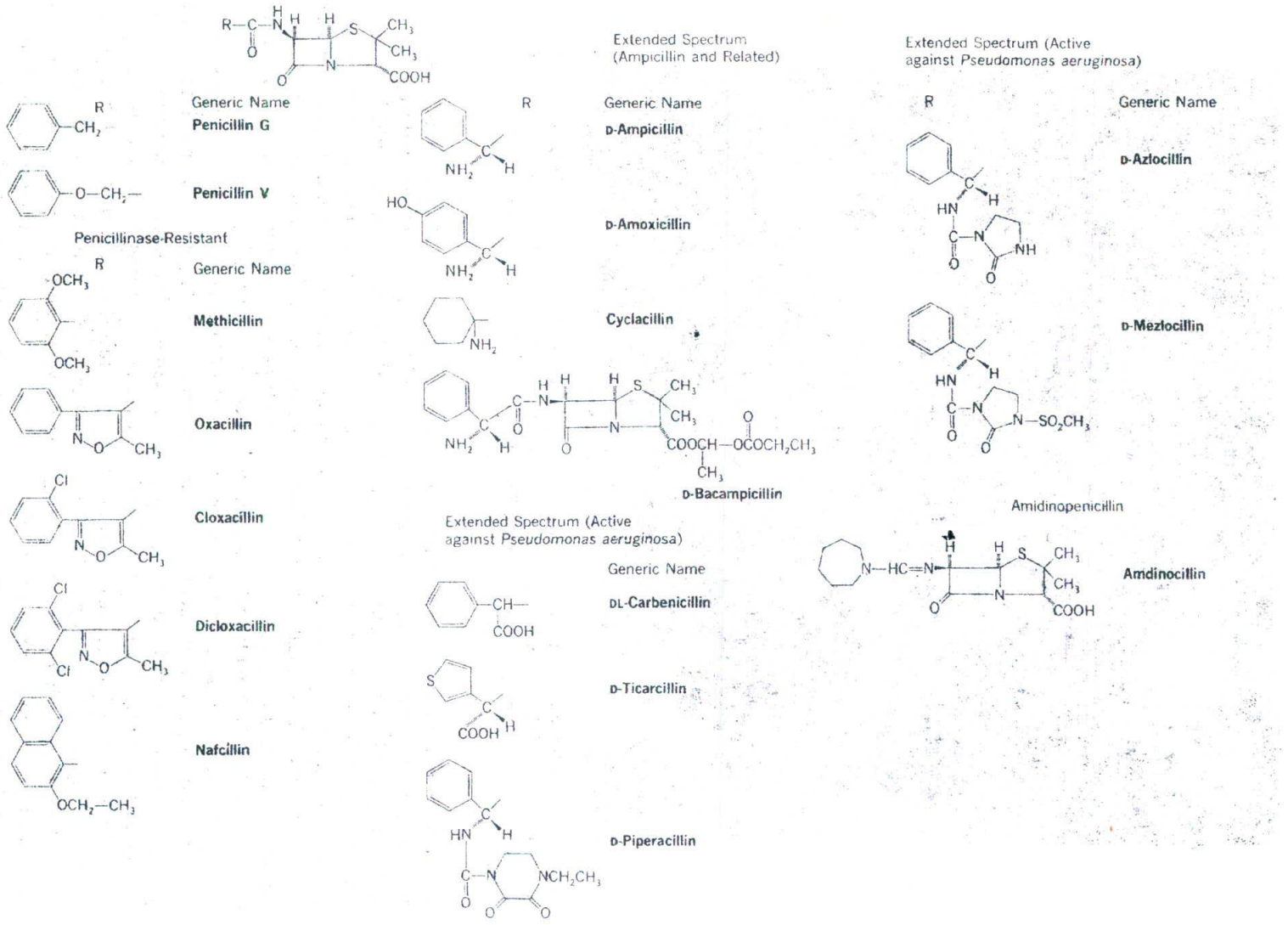


Fig. 12–13. Structures of commercially available penicillins.

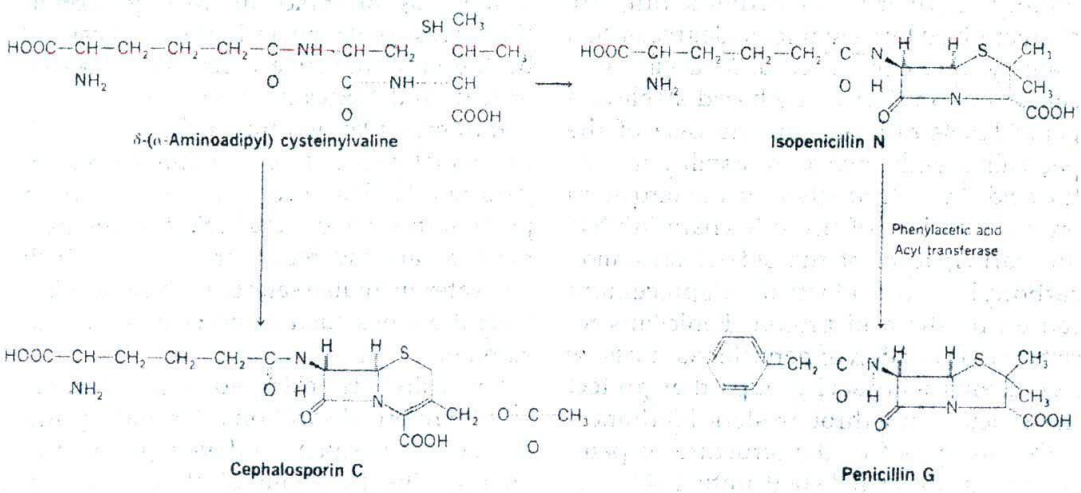


Fig. 12–14. Biosynthesis of penicillin G and cephalosporin C.

Unfortunately, the 4-membered β-lactam ring has considerable biologic and chemical lability, which has created a number of problems in the therapeutic utilization of these antibiotics. Biologically, microorganisms resistant to the action of penicillin G produce a β-lactamase (penicillinase) which hydrolyzes the β-lactam ring to form inactive penicilloic acid (Fig. 12–15). Chemically, penicillin G is rapidly inactivated when the pH is more acid than 5.0 or more alkaline than 8.0. In acidic conditions, penicillin G is converted to penillic acid and to penicilloic acid in alkali (Fig. 12–15). Ideally, aqueous solutions of penicillin G salts should be buffered at pH 6.8 for maximum stability. Penicillins are also inactivated by metal ions, such as zinc and copper, and by oxidizing agents. The need for penicillin antibiotics with inherent stability in gastric fluids and with resistance to penicillinase prompted the search for and development of other penicillins. Penicillin V, ampicillin, and the penicillins chemically related to ampicillin (amoxicillin, bacampicillin, cyclacillin), as well as the

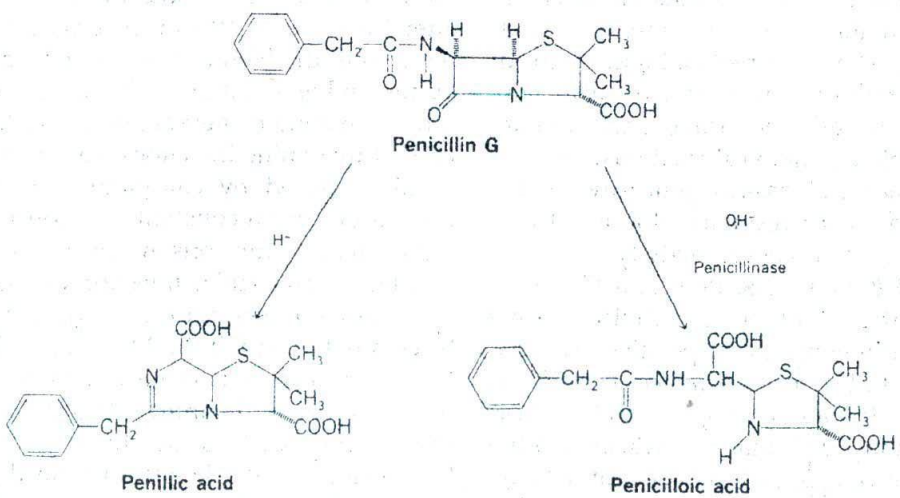


Fig. 12–15. Hydrolysis of the β-lactam system of penicillin G.

indanyl derivative of carbenicillin, are characterized by a significant degree of acid stability, and cloxacillin, dicloxacillin, nafcillin, and oxacillin are both acid-stable and penicillinase-resistant. In the case of the penicillins with increased stability in gastric acid, the introduction of a hetero atom on the α-carbon of the side chain inhibits the participation of the side-chain amide carbonyl in the electron displacement caused by the acid proton. Penicillins resistant to the action of penicillinase possess side chains with acyl groups that protect the β-lactam ring through steric hindrance.

Definite proof of the structure of penicillin was not established until 1949. The need for quantitation of penicillin antibiotics prior to complete elucidation of their chemistry and prior to feasible approaches for resolving or avoiding mixtures of penicillins resulted in the use of microbiologic assay. Microbiologic assay still has some utility in evaluating certain antibiotics, and biologic units are used to express quantitation for penicillin G and penicillin V. Sodium penicillin G is currently accepted as the reference standard; 1 unit is the antibiotic activity of 0.6 μg of sodium penicillin G reference standard. Microbial assays must be conducted under carefully controlled conditions, and alternate procedures frequently offer some advantages at the present time for quantitation of chemical availability. However, therapeutic efficacy is best determined biologically. In addition, a reduction in antimicrobial activity will reveal subtle chemical changes not demonstrable by chemical methods. Accordingly, microbial assays generally remain the standard for resolving doubt with respect to possible loss of activity.

USE OF PENICILLINS. Penicillin G is considered the agent of first choice against many pathogenic gram-positive bacteria. These include *Bacillus anthracis, Clostridium tetani, Clostridium perfringens, Staphylococcus aureus* (nonpenicillinase-producing), β-hemolytic group A *Streptococcus*, and *Streptococcus pneumoniae*. In addition, penicillin is the drug of choice in treating syphilis *(Treponema pallidum)* and infections caused by the gram-negative cocci, *Neisseria gonorrhoeae* and *Neisseria meningitidis*.

Intramuscular or intravenous injection is the usual method of administration for penicillin G. The water-soluble sodium or potassium salts are available for this purpose, as are the repository forms, which are water-insoluble salts of high-molecular-weight amines such as procaine and benzathine.

Penicillin G is destroyed by gastric acid, and therefore absorption after oral administration is irregular and variable; consequently, the penicillin of choice for oral administration is penicillin V, which is less susceptible than penicillin G to degradation by gastric acid and produces blood levels 2 to 5 times higher than penicillin G. The patient should also be cautioned to take the antibiotic on an empty stomach (1 hour before or 2 hours after eating), because food inhibits its absorption.

In vitro sensitivity tests have shown that strains of group A *Streptococcus* have a sensitivity to penicillin G as low as 0.006 μg per ml; and the MIC range for other bacteria causing infections in which penicillin G is recommended is 0.01 to 2.0 μg per ml. The rapid intravenous administration of sodium or potassium penicillin G results in an immediate high blood level; however, after 1 hour, only 10% of the original dose remains in the blood owing to both distribution and elimination of the drug. Therefore, to maintain therapeutic blood levels in life-threatening infections, the antibiotic is administered by continuous infusion, preferably with a constant infusion pump rather than by the constant drip method.

After intramuscular injection of sodium or potassium penicillin G, a peak blood level is obtained within 30 minutes. Then the serum level falls rapidly, with a usual half-life of only 30 minutes. It is important to remember that the height of the blood level peak and the length of time during which penicillin may be demonstrated in

the blood depend on the dose and also can vary from person to person.

In order to maintain therapeutic blood levels of penicillin G, 2 approaches are utilized. One approach interferes with the excretion of the antibiotic in the tubules of the kidney. When probenecid is used with penicillin, penicillin serum concentrations are approximately doubled. The supposed mechanism involves the hydrolysis of probenecid in the body to yield benzoic acid, which is conjugated in the liver with glycine to provide β-aminohippuric acid, which, in turn, competes with penicillin for renal excretion.

A more widely used approach for maintaining therapeutic blood levels delays absorption by employing repository penicillins. After intramuscular injection of an aqueous suspension of procaine penicillin G, a peak blood level is obtained in about 2 hours, and if an adult dose of at least 600,000 units is used, detectable levels are maintained in most patients for at least 24 hours. Benzathine penicillin G is less water-soluble than the procaine salt, and, following intramuscular injection of 600,000 units in an aqueous suspension, serum concentrations of 0.018 to 0.06 μg per ml persist for up to 2 weeks.

Resistance to penicillin G occurs fairly frequently among strains of *Staphylococcus aureus*. For this reason, if it is not known whether the infecting organism is a penicillinase producer, a penicillinase-resistant semisynthetic penicillin is normally the antibiotic of first choice until a culture-sensitivity test can be performed. Among the penicillinase-resistant semisynthetic penicillins exists essentially equivalent antimicrobial activity against pathogenic gram-positive cocci with MICs of 0.05 to 1.0 μg per ml; however, in general they are less effective than penicillin G against these organisms.

Extended spectrum penicillins have activity against certain pathogenic gram-negative bacilli against which penicillin G has little activity at normal therapeutic doses. Based on microbial activity, these extended spectrum penicillins can be divided into two groups. One group, composed of ampicillin and the chemically related compounds amoxicillin, bacampicillin, and cyclacillin, is useful in the treatment of *Escherichia coli, Haemophilus influenzae, Salmonella,* and *Shigella* infections, as well as of those infections caused by gram-negative cocci and gram-positive organisms. The second group, which includes carbenicillin, ticarcillin, mezlocillin, piperacillin, and azlocillin, is important in treating *Enterobacter, Escherichia coli, Bacteroides, Proteus,* and *Pseudomonas aeruginosa* infections. In addition, mezlocillin, piperacillin, and azlocillin are indicated against *Klebsiella pneumoniae*. The most recently marketed extended-spectrum penicillin, amdinocillin, has been approved for use in urinary tract infections caused by *Escherichia coli, Klebsiella,* and *Enterobacter.*

A possible explanation for the extension of the antimicrobial spectrum of some of the penicillins such as ampicillin is that these antibiotics penetrate to the site of action more readily than does penicillin G. The mucopeptide layer of the cell wall of gram-negative organisms lies behind layers of polysaccharide, protein, and lipid, which serve as a penetration barrier to penicillin G but not to the extended spectrum penicillins. In the wild type *Escherichia coli* with an intact penetration barrier, the MIC for penicillin G is 200 μg per ml as opposed to 2 μg per ml for ampicillin; however, if the penetration barrier is removed through mutation, penicillin G exhibits an MIC of 5 μg per ml and ampicillin an MIC of 0.5 μg per ml against the mutant strain.

The penicillins act by inhibiting mucopeptide formation in bacterial cell walls (see page 325). Presumably, the lack of comparable metabolism in zoologic systems contributes to the relatively low incidence of serious side effects with these antibiotics. The most frequent adverse reactions are allergic responses, and the occasional incidence of anaphylactic shock

can be fatal in the absence of emergency treatment. Epinephrine is usually administered for symptomatic control in penicillin shock.

In the early years of penicillin therapy, many cases of hypersensitivity were attributed correctly to foreign proteins, and the frequency of such reactions was reduced by improved purification procedures. However, impurities are not responsible for all penicillin reactions. Penicillin acts as a hapten and combines with body proteins to form an antigen which, in this case, is an allergen. Cross-sensitivity occurs among all compounds with the penicillin nucleus; however, there is experimental and clinical evidence that some semisynthetic penicillins are less allergenic. Sensitization is usually owing to a previous treatment with penicillin, but some people get an allergic reaction when first treated owing to a hidden contact such as consumption of milk containing penicillin as a result of veterinary treatment. A history of hypersensitivity to penicillin is an indication to use alternate antibiotics for control of penicillin-susceptible pathogens.

Penicillin G potassium or potassium benzyl penicillin is normally formulated with suitable buffer systems. Solid formulations have an expiration date that is not later than 5 years from the time the lot was released by the manufacturer. Sterile aqueous solutions may be stored in a refrigerator for 3 to 7 days (the latter if an approved sodium citrate buffer is used) without significant loss of potency, and the appropriate storage period must be stated on the label.

Penicillin G potassium may be administered orally, intramuscularly, or intravenously. One mg of pure penicillin G potassium is equivalent to 1595 units. The usual dose is 200,000 to 500,000 units, 3 or 4 times daily orally, and 500,000 to 1 million units intravenously, 6 to 8 times a day. Daily doses of 10 million units or more are given by intravenous infusion, and up to 100 million units daily may be administered by this method. The dose range varies widely, depending on the pathogen being treated, the extent of the infection, and other clinical conditions.

PRESCRIPTION PRODUCTS. Numerous preparations of penicillin G potassium are available, including Pentids® and Pfizerpen G®.

Penicillin G sodium or sodium benzyl penicillin is normally formulated with suitable buffer systems. Solid preparations have an expiration date that is not later than 5 years from the time the lot was released by the manufacturer, and sterile aqueous solutions may be stored in a refrigerator for 3 days without significant loss of potency. Pure penicillin G sodium is used as the reference standard for microbial assays of the penicillins, and 1 mg is equivalent to 1667 units. Penicillin G sodium is used orally, intramuscularly, or intravenously in the same manner as the potassium salt. A wide range in dosage will be encountered; the usual dose is considered to be 400,000 units, 4 times a day, orally or intramuscularly, and 10 million units daily, intravenously.

Penicillin G procaine is the slightly soluble procaine salt of penicillin G. One mg of pure penicillin G procaine is equivalent to 1009 units.

This antibiotic is used intramuscularly and has the advantage of prolonged action because of slow absorption. It is formulated in an aqueous suspension. The usual intramuscular dose is 300,000 to 600,000 units, 1 or 2 times a day.

PRESCRIPTION PRODUCTS. Crysticillin A. S.®, Duracillin A. S.®, Wycillin®, Pfizerpen-A.S.®.

Penicillin G benzathine or *N,N'*-dibenzylethylenediamine dipenicillin G is a slightly soluble salt of penicillin that is used intramuscularly for its unusually prolonged duration of action and orally for its resistance to gastric inactivation. One mg of pure penicillin G benzathine is equivalent to 1211 units, and commercial material must have a potency of not less than 1050

units per mg. The usual dose, intramuscularly, is 1.2 million to 2.4 million units as a single dose or 600,000 to 1.2 million units, 2 times a month to 3 times a week. The oral dose is 400,000 to 600,000 units, 4 to 6 times a day.

PRESCRIPTION PRODUCTS. Bicillin®, Permapen®.

Penicillin V or phenoxymethyl penicillin and penicillin V potassium or phenoxymethyl penicillin potassium are relatively acid-stable. The potassium salt is soluble in water, and this appears to favor better absorption following oral administration. However, both forms of this penicillin gave good blood levels, the average ranging from 2 to 5 times higher than the levels obtainable with comparable oral doses of penicillin G. One mg of pure phenoxymethyl penicillin is equivalent to 1695 units, and 1 mg of the pure potassium salt equals 1530 units. Quantities are usually expressed on a weight basis for preparations of these penicillins, and the usual oral dose is 125 to 500 mg (200,000 to 800,000 units), 3 or 4 times a day.

PRESCRIPTION PRODUCTS. Ledercillin VK®, Pen-Vee K®, V-Cillin K®, Veetids®, Pfizerpen VK®.

Penicillinase-Resistant Penicillins

Cloxacillin, dicloxacillin, methicillin, nafcillin, and oxacillin are semisynthetic penicillins that are not inactivated by penicillinase. They are recommended primarily for treatment of staphylococcal infections resistant to other penicillins. Methicillin is acid-labile and must be administered parenterally. The other 4 penicillins are stable in gastric acidity. Nafcillin and oxacillin are available in formulations for oral and parenteral administration; cloxacillin and dicloxacillin are only used orally. All of these penicillins are employed as sodium salts.

Cloxacillin, dicloxacillin, nafcillin, and oxacillin are characterized by a high degree of binding to serum protein, but approximately only 20% of methicillin in blood is bound to serum proteins. Dicloxacillin is absorbed more readily than the other penicillinase-resistant penicillins administered orally. However, food interferes sufficiently with the absorption of all these penicillins, including dicloxacillin, to prompt the recommendation that they be administered on a fasting stomach in order to obtain satisfactory blood levels. A 500-mg oral dose of oxacillin results in a peak blood level of 2.6 μg per ml in 1 hour. When compared with oxacillin at the same dose, cloxacillin provides a 2-fold higher blood level, and dicloxacillin effects a 4 times higher blood level. The usual doses are 1 g, intramuscularly or intravenously, 4 to 6 times a day for methicillin; 250 mg to 1 g, orally, 4 to 6 times a day; and 500 mg to 1 g, intramuscularly or intravenously, 4 to 6 times a day for nafcillin; 500 mg to 1 g, orally, 4 to 6 times a day and 250 mg to 1 g, intravenously or intramuscularly, 4 to 6 times a day for oxacillin; 250 to 500 mg, orally, 4 times a day for cloxacillin; and 125 to 250 mg, orally, 4 times a day for dicloxacillin.

PRESCRIPTION PRODUCTS. Cloxacillin: Tegopen®; dicloxacillin: Dynapen®, Pathocil®, Veracillin®; methicillin: Staphcillin®, Celbenin®; nafcillin: Unipen®, Nafcil®; oxacillin: Prostaphlin®, Bactocill®.

Extended-Spectrum Penicillins (Ampicillin-Related)

Ampicillin or aminobenzyl penicillin is an acid-stable, readily absorbed semisynthetic penicillin. It is inactivated by penicillinase but has an unusual spectrum of activity for a penicillin. It is active against most of the bacteria sensitive to penicillin G but also has greater activity against certain gram-negative bacilli than penicillin G. The L-isomer of ampicillin is only about as active as penicillin G against gram-negative bacteria. The D-isomer shows increased activity; therefore, the D-isomer is used in therapy. Ampicillin has special clinical value for treatment of infections caused by *Haemophilus influenzae*, *Salmonella* species,

and *Shigella* species. This antibiotic effectively controls nonpenicillinase-forming strains of *Proteus mirabilis* and *Escherichia coli*, but the high frequency of penicillinase formation by these pathogens limits the clinical effectiveness of ampicillin with respect to these species. The in vitro MICs for ampicillin range from 0.25 μg per ml for strains of *Haemophilus influenzae* to 5 μg per ml for sensitive strains of *Escherichia coli*. A 250-mg oral dose of ampicillin results in a peak blood level of 1.8 μg per ml reached in 2 hours; therefore, it is important to use high doses to treat *Escherichia coli* infections. The sodium salt of ampicillin is used in parenteral formulations, and oral dosage forms normally utilize the free acid. The usual dose is 250 to 500 mg, orally, 4 times a day and 500 mg, intramuscularly or intravenously, 4 times a day.

PRESCRIPTION PRODUCTS. Amcill®, Omnipen®, Polycillin®, Principen®, Totacillin®.

Amoxicillin is the *p*-hydroxy derivative of ampicillin. It is stable in the presence of gastric acid and better absorbed from the gastrointestinal tract in the presence of food than ampicillin. It also produces less gastrointestinal disturbance than ampicillin, and since it has antibacterial activity similar to that of ampicillin, it is rapidly replacing this drug in therapeutics. The Centers for Disease Control recommends amoxicillin as the agent of choice in the primary treatment of uncomplicated gonorrhea. It is administered in a 3-g single oral dose along with 1 g probenecid (to slow renal excretion of the amoxicillin), followed by 500 mg of tetracycline 4 times daily for 7 days. This regimen has the advantage of single-dose effectiveness against gonorrhea, combined with effectiveness against *Chlamydia trachomatis* which is also often present in patients with gonorrhea and is the most common cause of postgonococcal urethritis. A 250-mg oral dose gives a peak blood level of 4.3 μg per ml. The usual dose is 250 to 500 mg, orally, 3 times a day.

PRESCRIPTION PRODUCTS. Amoxil®, Larotid®, Polymox®, Trimox®, Utimox®, Wymox®.

Bacampicillin hydrochloride is hydrolyzed to ampicillin during absorption from the gastrointestinal tract. Because it is more completely absorbed than ampicillin, it is administered in lower total daily dosages, with 400 mg chemically equivalent to 280 mg of ampicillin, and it sustains effective serum levels when given every 12 hours. Food does not interfere with the absorption from the tablets, but the suspension should be administered on a fasting stomach. The usual dose is 400 to 800 mg every 12 hours.

PRESCRIPTION PRODUCT. Spectrobid®.

Cyclacillin is acid-stable and rapidly and well absorbed from the gastrointestinal tract. The peak concentration after a 500-mg dose is 4 times higher than that of ampicillin and about 1.5 times the peak concentration of oral amoxicillin. Although cyclacillin has an antimicrobial spectrum similar to that of ampicillin, in vitro it has less activity than ampicillin against many organisms. It has been approved for treatment of respiratory tract infections, otitis media, skin infections caused by gram-positive cocci and *Haemophilus influenzae*, and urinary tract infections caused by *Escherichia coli* and *Proteus mirabilis*. The usual dose range is 250 to 500 mg 4 times daily.

PRESCRIPTION PRODUCT. Cyclapen-W®.

Extended-Spectrum Penicillins (Active Against *Pseudomonas aeruginosa*)

Carbenicillin disodium is a carboxybenzyl penicillin with increased antibacterial activity against gram-negative bacilli. The D- and L-isomers display only slight differences in biologic activity and undergo rapid interconversion when in solution; therefore, the racemic mixture is used. Carbenicillin is a drug of choice in the treatment of *Pseudomonas aeruginosa* infections and is an alternate antibiotic in *Escherichia coli*, *Enterobacter*, and *Proteus* infections. The antibiotic can be administered in sufficient dosage (up to 40 g daily) to obtain

serum concentrations exceeding 50 to 60 μg per ml. Such concentrations inhibit most *Pseudomonas aeruginosa* strains. Clinical efficacy may be enhanced by combination therapy of carbenicillin disodium with gentamicin or tobramycin in full therapeutic dosages. Carbenicillin is particularly effective in urinary tract infections because of very high urine levels achieved by intramuscular injection. The usual dose, intramuscularly or intravenously, is the equivalent of 1 to 2 g of carbenicillin, 4 times a day and, by intravenous infusion, up to 40 g a day. Carbenicillin indanyl sodium is also available for oral administration. The usual dose is 382 to 764 mg, 4 times a day.

PRESCRIPTION PRODUCTS. Carbenicillin disodium: Geopen®, Pyopen®; carbenicillin indanyl sodium: Geocillin®.

Ticarcillin disodium is the thienyl analog of carbenicillin and has the same antimicrobial spectrum and indications. Evidence suggests that it is somewhat more active than carbenicillin, particularly against *Pseudomonas aeruginosa*. It is also used in combination with gentamicin and tobramycin. The usual dose for uncomplicated urinary tract infections is 1 g every 6 hours, either intramuscularly or intravenously. For urinary tract infections with complications, the intravenous dose is 150 to 200 mg per kg per day in divided doses every 4 to 8 hours. For systemic infections, the adult intravenous dose is 200 to 300 mg per kg of body weight daily in divided doses every 3, 4, or 6 hours.

PRESCRIPTION PRODUCT. Ticar®.

Mezlocillin sodium is an ureidopenicillin similar to carbenicillin and ticarcillin in its antibacterial spectrum. It is active against gram-positive cocci, and most strains of *Haemophilus influenzae* and gonococcus are highly susceptible to mezlocillin. It is more active in vitro than carbenicillin and ticarcillin against susceptible strains of enteric gram-negative bacilli such as *Escherichia coli*, *Klebsiella*, and *Enterobacter*. The activity of mezlocillin against *Pseudomonas aeruginosa* is comparable to that of ticarcillin. It is available for intravenous or intramusuclar use. For life-threatening infections, it should be given intravenously in a dose of 4 g every 4 hours.

PRESCRIPTION PRODUCT. Mezlin®.

Piperacillin sodium is an aminobenzylpenicillin derivative with an antibacterial spectrum similar to that of mezlocillin. It is active in vitro against gram-positive cocci, enteric gram-negative bacilli, and many anaerobes. It is less active than penicillin G against pneumococci and group A β-hemolytic streptococci. It is more active in vitro than carbenicillin, ticarcillin, or ampicillin against *Escherichia coli*, *Klebsiella*, and *Enterobacter;* however, it is 4 to 16 times more active than carbenicillin, ticarcillin, or mezlocillin against *Pseudomonas aeruginosa*. Piperacillin is available for intramuscular or intravenous use. For serious infections, the maximum dose is 3 to 4 g every 4 to 6 hours. No more than 2 g should be given intramuscularly at any one site.

PRESCRIPTION PRODUCT. Pipracil®.

Azlocillin sodium is an ureidopenicillin with a spectrum of activity similar to those of mezlocillin and piperacillin. Against *Pseudomonas aeruginosa*, it is more active in vitro than carbenicillin, ticarcillin, or mezlocillin, and it is similar to piperacillin. After equal doses, serum levels of azlocillin are higher than those obtained with mezlocillin or ticarcillin. Since azlocillin, mezlocillin, and piperacillin are all monosodium salts, these agents are less likely to cause fluid retention than carbenicillin or ticarcillin, which are disodium salts. For serious infections in adults, 3 to 4 g of azlocillin can be given every 4 to 6 hours up to a maximum of 24 g daily.

PRESCRIPTION PRODUCT. Azlin®.

Amidinopenicillin

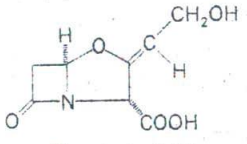
Amdinocillin represents a new class of β-lactam antibiotics. It is a semisynthetic 6-amidino-penicillanic acid. Since it contains the β-lactam-thiazolidine fused ring structure, it can be considered a close relative of the penicillins. This change in the chem-

ical structure, however, is associated with a marked alteration in its antibacterial spectrum and mechanism of action. Amdinocillin is considerably more active against gram-negative bacilli than against gram-positive cocci, whereas the reverse is true for penicillin G. Amdinocillin interferes with bacterial wall synthesis in *Escherichia coli* by binding principally to penicillin binding protein 2 (PBP-2). Other β-lactam antibiotics bind mostly to PBPs-1 and -3, which suggests that concurrent use of amdinocillin with other β-lactams might have a synergistic effect on susceptible bacteria. The recommended dosage is 10 mg per kg every 4 to 6 hours intravenously or intramuscularly.

PRESCRIPTION PRODUCT. Coactin®.

Penicillins Combined with Clavulanic Acid

Clavulanic acid is a fermentation product of *Streptomyces clavuligerus*, structurally related to the penicillins. Although only weakly antibacterial on its own, clavulanic acid is capable of irreversibly inactivating bacterial β-lactamases responsible for antibiotic resistance. Clavulanic acid acts synergistically with β-lactamase–sensitive penicillins and cephalosporins, and clavulanic acid concentrations of 5 μg per ml or less may decrease the in-vitro MICs of these antibiotics against bacteria normally resistant to therapeutically useful levels. The synergy mainly results from the protection afforded to the sensitive antibiotic caused by the inactivation of β-lactamase by clavulanic acid.



Clavulanic Acid

The combination of **amoxicillin and potassium clavulanate** is a β-lactam antibiotic with a β-lactamase inhibitor. The addition of clavulanic acid extends the in vitro activity of amoxicillin to include β-lactamase–producing strains of *Haemophilus influenzae*, *Escherichia coli*, *Proteus*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. The combination is available in tablets for oral use containing 250 and 500 mg of amoxicillin. Each tablet strength contains 125 mg of potassium clavulanate; therefore, two 250-mg tablets are not equivalent to one 500-mg tablet, and the higher dose of clavulanic acid is more likely to cause diarrhea. The usual adult dosage is 250 mg every 8 hours, and 500 mg every 8 hours is recommended for severe infections.

PRESCRIPTION PRODUCT. Augmentin®.

A combination product of **ticarcillin disodium and potassium clavulanate** is available for parenteral treatment of urinary tract, skin and soft tissue, and lower respiratory tract infections, and sepsis due to susceptible organisms. When clavulanic acid is added to ticarcillin, a striking increase in activity occurs against β-lactamase–producing strains of *Staphylococcus aureus*, *Haemophilus influenzae*, gonococcus, *Escherichia coli*, and *Klebsiella*. The usual adult dosage is 3 g of ticarcillin and 100 mg of clavulanic acid every 4 to 6 hours administered by intravenous infusion over 30 minutes.

PRESCRIPTION PRODUCT. Timentin®.

Cephalosporins and Other β-Lactam Antibiotics

In 1945, Brotzu isolated a microorganism from sea water collected near a sewage outlet off the coast of Sardinia and noted its antagonism to both gram-positive and gram-negative bacteria. The organism was identified as *Cephalosporium acremonium*. Abraham and his coworkers at Oxford reported the isolation of 3 substances with antibiotic activity from cultures of this organism during 1955 and 1956. These metabolites were a steroid (cephalosporin P) that has achieved no therapeutic significance, penicillin N, and cephalosporin C. Cephalosporin C is biosynthetically related to the penicillins (see page 333) and resembles these antibiotics in many of its biologic

and chemical properties. The major difference is a 7-aminocephalosporanic acid nucleus which has a fused dihydrothiazine β-lactam ring system rather than the fused thiazolidine β-lactam system of 6-aminopenicillanic acid. The degree of antibacterial activity of cephalosporin C is only moderate, and it is not used therapeutically. However, it is produced by fermentation in large quantities to serve as a starting material for the chemical production of the semisynthetic cephalosporin antibiotics (Fig. 12–16).

The cephamycins are β-lactam antibiotics that are closely related chemically to the cephalosporins. They are produced by actinomycetes rather than fungi and are 7-α-methoxycephalosporins. Cephamycin C, which is produced by *Streptomyces lactamdurans*, serves as a starting material for the chemical synthesis of cefoxitin, the only cephamycin antibiotic currently available for therapeutic use.

A new group of antibiotics related to the cephalosporins has been obtained through partial synthesis from penicillin. In these antibiotics the dihydrothiazine ring of the cephalosporins has been replaced by a dihydrooxazine ring. Apparently the sulfur atom in the cephalosporin ring system is not a requirement for high antibiotic activity. These antibiotics have been designated oxalactams. In this group, moxalactam is a 7-α-methoxyoxalactam derivative in which the methoxy group protects the oxalactam nucleus from β-lactamase hydrolysis.

Imipenem is another new type of β-lactam antibiotic. It is a member of a class of antibiotics containing the carbapenem nucleus in which the sulfur atom of penicillin has been replaced by a carbon atom. Thienamycin, which is a naturally occurring carbapenem compound produced by *Streptomyces cattleya*, serves as the starting material for the synthesis of imipenem (*N*-formimidoylthienamycin).

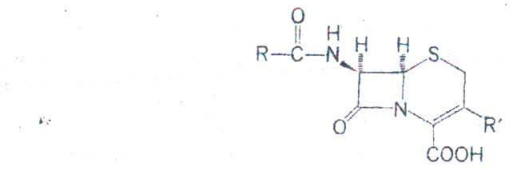
The cephalosporins and related antibiotics can be divided into first, second, and third generation agents based on in-vitro antibacterial activity. The first generation cephalosporins, such as cephalothin and cefazolin, are active against most gram-positive cocci, *Haemophilus influenzae*, and some strains of gram-negative enteric bacilli, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. The second generation group, which includes, among others, the cephalosporins cefamandol and cefuroxime, as well as cefoxitin, a cephamycin, is more active against *Haemophilus influenzae*, gonococcus, enteric gram-negative bacilli, and some strains of anaerobes. The third generation group, which includes moxalactam (an oxalactam), imipenem (a carbapenem), and many cephalosporins, is active against enteric gram-negative bacilli, gonococcus, meningococcus, and anaerobes such as *Bacteroides fragilis*; it has varying effectiveness against *Pseudomonas aeruginosa*.

In general, progression from first to third generation drugs reveals a broadening gram-negative spectrum, a loss of efficacy against gram-positive organisms, and a greater efficacy against resistant organisms. First generation drugs are generally inactivated by β-lactamase–producing organisms. Second and third generation agents are distinguished by an increasing resistance to β-lactamase inactivation.

The cephalosporins and related β-lactam antibiotics inhibit cell-wall formation, and this general mode of action explains their relatively low toxicity. Hypersensitivity is a frequent side effect of the cephalosporins. Some cross-sensitivity reactions in patients allergic to penicillin necessitate care in administering cephalosporins to individuals who are allergic to penicillin. Pseudomembranous colitis has been reported with the use of cephalosporins.

First Generation Agents

Cephalothin, cefazolin, cephapirin, cephradine, cephalexin, cefadroxil, and cefaclor have antibacterial activity similar to that of ampicillin. They are effective against gram-positive bacteria, including



Cephalosporin Nucleus

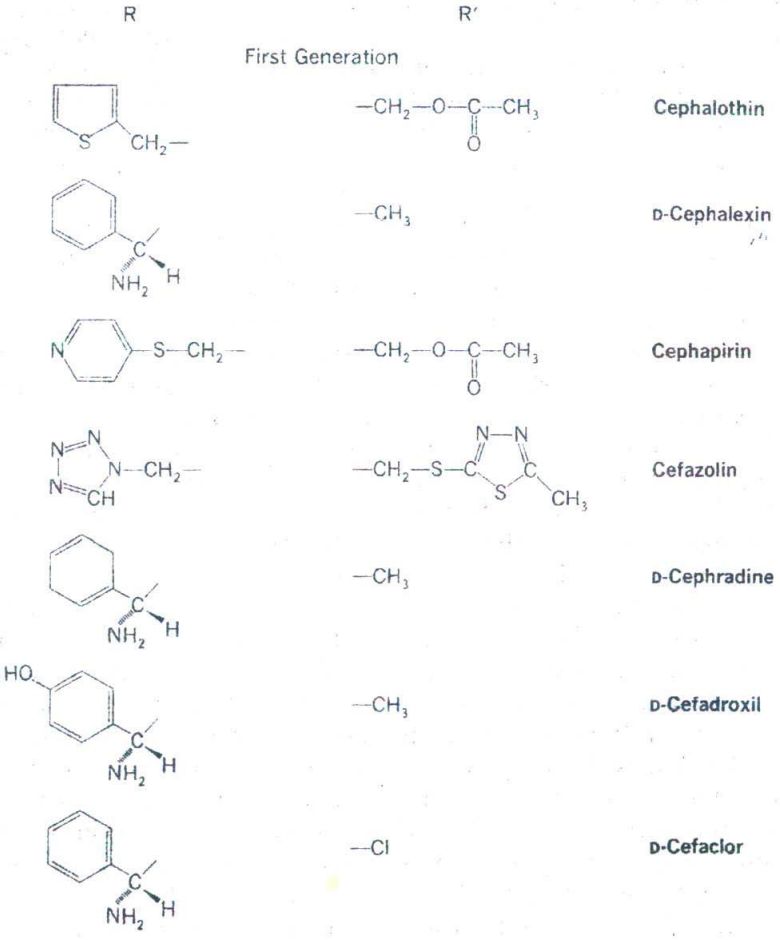


Fig. 12–16. Structures of commercially available cephalosporins and other β-lactam antibiotics.

Second Generation

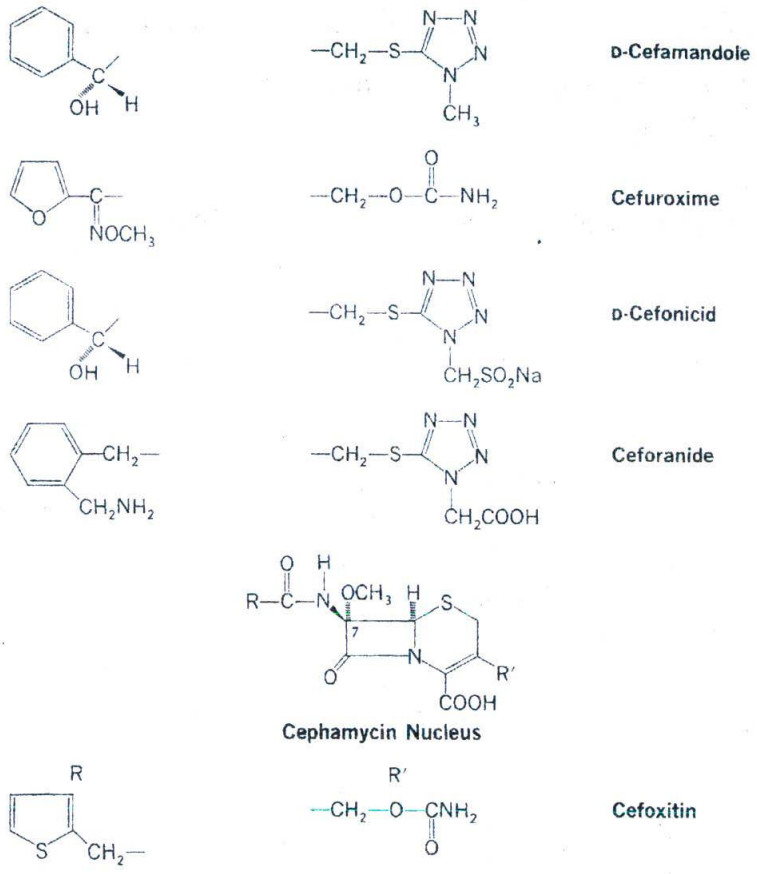
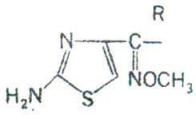
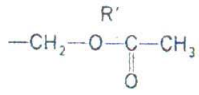
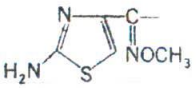


Fig. 12–16. *Continued*

Third Generation

Cefotaxime



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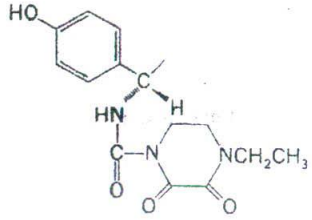
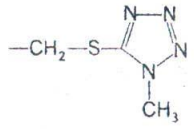
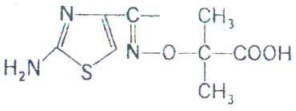
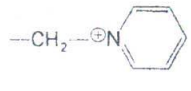
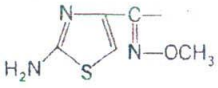
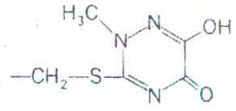
Ceftizoxime

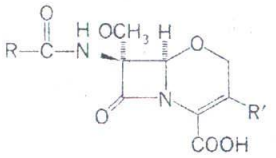
D-Cefoperazone

Ceftazidime

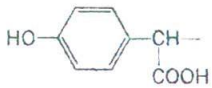
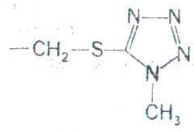



Ceftriaxone

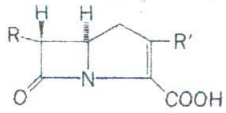


Oxalactam Nucleus

R R′

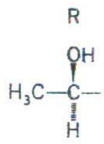



DL-Moxalactam



Carbapenem Nucleus

R R′



—S—CH_2CH_2—NH—CH=NH **Imipenem**
[(+)-*N*-formimidoyl-thienamycin]

Fig. 12–16. *Continued*

penicillinase-producing *Staphylococcus* (Table 12–3). The first generation cephalosporin antibiotics are resistant to penicillinase, but they are inactivated by another β-lactamase, cephalosporinase. Certain gram-negative organisms, including *Haemophilus influenzae, Escherichia coli, Proteus mirabilis,* and *Klebsiella pneumoniae,* are sensitive to these cephalosporins. Species of *Enterobacter* and *Pseudomonas* as well as most indole-producing species of *Proteus* are resistant to this group. The susceptibility of *Haemophilus influenzae* is quite variable, and, generally, the first generation cephalosporins are less active against this organism than are the extended-spectrum penicillins such as ampicillin. The cephalosporins penetrate most tissues well except that, unlike the penicillins, they are unpredictable in the manner in which they cross the blood-brain barrier; consequently, the first generation cephalospo-

Table 12–3. Therapeutically Important Cephalosporin and Other β-Lactam Antibiotics

Generic Name	Prescription Products	Usual Dose	Peak Blood Levels
First Generation:			
Cephalothin sodium	Keflin®	500 mg to 1 g every 4 to 6 hours parenterally[a]	30–64 μg/ml[b]
Cefazolin sodium	Kefzol® Ancef®	500 mg to 1.5 g every 6 to 8 hours parenterally[a]	185–189 μg/ml[b]
Cephapirin sodium	Cefadyl®	500 mg to 1 g every 4 to 6 hours parenterally[a]	40–73 μg/ml[b]
Cephradine	Velosef® Anspor®	500 mg to 1 g 4 times daily parenterally[a] or	86 μg/ml[b]
		250 mg every 6 hours or 500 mg every 12 hours orally	9 μg/ml[c]
Cephalexin	Keflex®	250 mg every 6 hours orally	9 μg/ml[c]
Cefadroxil	Duricef® Ultracef®	500 mg to 1 g, 2 times daily orally	9 μg/ml[c]
Cefaclor	Ceclor®	250 mg every 8 hours orally	6 μg/ml[c]
Second Generation:			
Cefamandole nafate	Mandol®	500 mg to 1 g every 4 to 8 hours parenterally[a]	88–139 μg/ml[b]
Cefuroxime sodium	Zinacef®	750 mg to 1.5 g every 8 hours parenterally[a]	43–98 μg/ml[b]
Cefonicid sodium	Monocid®	1 g per 24 hours parenterally[a]	221 μg/ml[b]
Ceforanide	Precef®	500 mg to 1 g every 12 hours parenterally[a]	125 μg/ml[b]
Cefoxitin sodium	Mefoxin®	1 to 2 g every 6 to 8 hours parenterally[a]	56–110 μg/ml[b]
Third Generation:			
Cefotaxime sodium	Claforan®	1 g every 6 to 8 hours parenterally[a]	81–102 μg/ml[b]
Ceftizoxime sodium	Cefizox®	1 to 2 g every 8 to 12 hours parenterally[a]	61–84 μg/ml[b]
Cefoperazone sodium	Cefobid®	1 to 2 g every 12 hours parenterally[a]	73–153 μg/ml[b]
Ceftazidime	Fortaz® Tazidime®	1 g every 8 to 12 hours parenterally[a]	69–90 μg/ml[b]
Ceftriaxone sodium	Rocephin®	1 to 2 g once daily parenterally[a]	151 μg/ml[b]
Moxalactam disodium	Moxam®	2 to 6 g per day in divided doses every 8 hours parenterally[a]	71–94 μg/ml[b]
Imipenem/cilastatin	Primaxin®	1 g every 6 hours intravenously	—

[a]Either intramuscularly or intravenously.
[b]1 g intravenous dose.
[c]1 hour after a 250-mg oral dose.

rins should never be considered an adequate substitute for the penicillins in treating meningitis. The MIC values for these antibiotics range from 0.007 μg per ml for strains of *Streptococcus* to 16 μg per ml for strains of *Haemophilus influenzae*. The first generation cephalosporins used orally, namely, cephalexin, cefadroxil, cefaclor, and cephradine (the last is also used parenterally), are less effective than the parenteral cephalosporins against *Escherichia coli, Proteus mirabilis,* and species of *Klebsiella*. The orally effective cephalosporins can be used to treat infections caused by gram-positive bacteria, and because of the high concentration of these antibiotics excreted in the urine, e.g., 1000 μg per ml following a 250-mg oral dose of cephalexin, they are used to treat urinary tract infections caused by *Escherichia coli, Proteus mirabilis,* and *Klebsiella* species. Because of a prolonged excretion, cefadroxil has the advantage of providing more sustained serum and urine concentrations than are obtained with other oral cephalosporins. Clinical studies indicate that cefadroxil, 1 g twice daily, is as effective as cephalexin, 500 mg 4 times daily. Cefaclor inhibits *Haemophilus influenzae,* including ampicillin-resistant strains, at an in-vitro MIC of 2 μg per ml, which makes it more active against this organism than are other available oral cephalosporins.

Cephalothin can cause thrombophlebitis when administered intravenously in doses larger than 6 g daily for periods longer than 3 days; also, intramuscular injection of this antibiotic may be painful. Cefazolin is the first cephalosporin marketed that seems to be devoid of these undesirable side effects.

Second Generation Agents

Cefamandole nafate, cefuroxime, cefonicid, ceforanide, and cefoxitin are classified as second generation agents. In general, they have the same spectrum of antibacterial activity as the first generation agents except that they are more active against *Haemophilus influenzae,* gonococcus, some enteric gram-negative bacilli, and anaerobes. The parenteral drugs cefamandole, cefuroxime, and cefonicid are about equally effective against *Haemophilus influenzae,* but ceforanide is less active. Cefuroxime and cefoxitin are effective against most strains of gonococcus, and meningococcus can be treated with cefuroxime because it reaches therapeutic concentrations in cerebrospinal fluid. Cefoxitin is indicated against the anaerobe *Bacteroides fragilis;* the other second generation agents are not.

Cefoxitin and cefuroxime have a high degree of resistance to many of the β-lactamases that can hydrolyze the commonly used cephalosporins. In the case of cefoxitin, this resistance is attributable to the steric hindrance around the 7 position of the cephamycin nucleus because of the 7-α-methoxy group. Cefuroxime has a methoxyimino group on its acyl side chain, which increases its resistance to β-lactamase.

The serum half-life of cefonicid is much longer than that of the other second generation agents, probably because of a high degree of protein binding. This allows for once a day dosing.

Cefamandole contains a methyltetrazolethiomethyl group at position 3 of the cephalosporin nucleus that has been associated with prothrombin deficiency and, sometimes, with bleeding. In addition, this group may inhibit aldehyde dehydrogenase, which may result in alcohol intolerance as a result of a disulfiram-like reaction.

Third Generation Agents

Cefotaxime, ceftizoxime, cefoperazone, ceftazidime, ceftriaxone, moxalactam, and imipenem/cilastatin are third generation agents. They are differentiated from first and second generation drugs by their extended activity against enteric gram-negative bacilli, including *Enterobacter,* and activity against some strains of *Pseudomonas aeruginosa*. Cefotaxime has superior in-

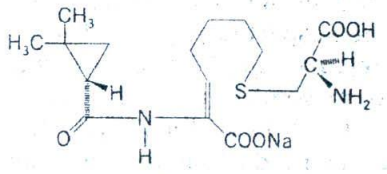
vitro activity against *Escherichia coli, Klebsiella, Enterobacter, Proteus, Haemophilus influenzae,* and *Neisseria,* with 90% of the strains of these organisms inhibited at an MIC of 0.5 μg per ml or less. The activity of cefotaxime against *Pseudomonas aeruginosa* is remarkable in comparison with other cephalosporins and carbenicillin, with an MIC of 8 μg per ml or less against 50% of the strains tested.

Ceftizoxime has a spectrum of activity very similar to that of cefotaxime, but it has a slightly longer half-life that permits 8- to 12-hour dosing as compared with 6 to 8 hours for cefotaxime. Cefoperazone is less active than cefotaxime against enteric gram-negative bacilli but more active against *Pseudomonas aeruginosa.* Ceftazidime has activity similar to that of cefotaxime and ceftizoxime in vitro but is more active against *Pseudomonas aeruginosa* and less active against staphylococci and *Bacteroides fragilis.* Ceftriaxone has only moderate activity against some strains of *Pseudomonas aeruginosa.* Most strains of meningococcus, gonococcus, and *Haemophilus influenzae* (including ampicillin-resistant strains) are highly susceptible to this antibiotic. Its longer half-life allows for once a day dosing.

Moxalactam has a spectrum of activity similar to that of cefotaxime; however, its use may be limited because of the occurrence of serious bleeding disorders. This agent as well as cefamandole and cefoperazone contains the methyltetrazolethiomethyl group, which may cause hypoprothrombinemia (see page 348).

Imipenem/cilastatin sodium is a fixed-dose combination product in a 1:1 ratio. Imipenem is a member of a class of antibiotics containing the carbapenem nucleus. It is prepared synthetically as the *N*-formimidoyl derivative of thienamycin, an antibiotic produced by *Streptomyces cattleya.*

When imipenem is administered alone, it is rapidly hydrolyzed to an inactive metabolite by dehydropeptidase-I which is present on the brush border of the proximal renal tubular cells; consequently, adequate antibacterial levels are not reached. This disadvantage has been overcome by the coadministration of the dehydropeptidase inhibitor cilastatin sodium, the sodium salt of a derivatized heptenoic acid.



Cilastatin

Data on both in-vitro and in-vivo studies suggest that imipenem/cilastatin inhibits 90% or more of the clinically important pathogens at an MIC of 8 μg per ml or less; therefore, clinical applications for this agent would be in infections resistant to other antibiotics and in the treatment of mixed infections that would otherwise require multiple antibiotics.

Chloramphenicol

Chloramphenicol was originally obtained from a culture of *Streptomyces venezuelae* Burkholder that was isolated in 1947 from a soil sample collected near Caracas, Venezuela. Because the organism had not been described previously, Burkholder applied the name *venezuelae* to the species. This antibiotic attracted considerable attention because it was the first truly broad-spectrum antibiotic discovered. Its spectrum of action includes gram-negative and gram-positive bacteria, a number of rickettsial pathogens, and a few viruses.

Chemically, chloramphenicol proved to be fairly simple. Its most unusual feature was the presence of a nitro group on a normal biologic metabolite. The molecular skeleton of the antibiotic suggested a biosynthetic origin via phenylpropanoid metabolism. Experimental studies with radioactive precursors have confirmed a shikimic acid-phenylpropanoid pathway for the biosynthesis of chloramphenicol, but the pathway apparently branches from normal phenylpropanoid metabolism prior

to the formation of phenylalanine or tyrosine. *p*-Aminophenylpyruvic acid has been suggested as an early metabolite in the biosynthetic pathway, and subsequent steps involving transamination, hydroxylation, acylation, reduction of the carboxyl group, and terminal oxidation of the amino group are suspected (Figure 12–17).

The relative simplicity of the chloramphenicol molecule led to the early development of feasible synthetic procedures for commercial production of the antibiotic. This antibiotic is unique with respect to the successful development of totally synthetic means for commercial production. Four isomers exist; the active one is D(−)-*threo*-2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide.

Chloramphenicol acts by inhibiting protein synthesis at the ribosome level. It binds preferentially to the 50S subunit of microbial 70S ribosomes and disrupts peptidyl transferase, the enzyme that catalyzes peptide bond formation. Other types of involvement can be detected experimentally, but their significance for therapeutic application of the antibiotic is unknown. The relative lack of affinity for mammalian 80S ribosomes presumably explains the low incidence of adverse reactions with chloramphenicol. However, occasional development of aplastic anemia (estimated to be 1:25,000 or less) and related blood dyscrasias, which may be irreversible and fatal, introduces a serious limitation on the therapeutic utility of this antibiotic. When chloramphenicol is employed, the blood picture should be monitored daily or on alternate days for evidence of changes in the reticulocytes or other abnormalities. Biochemical details of the toxicities have not been clarified fully, but microbial reduction by the gut flora of the nitro function in a small percentage of the ingested chloramphenicol may be a contributing factor.

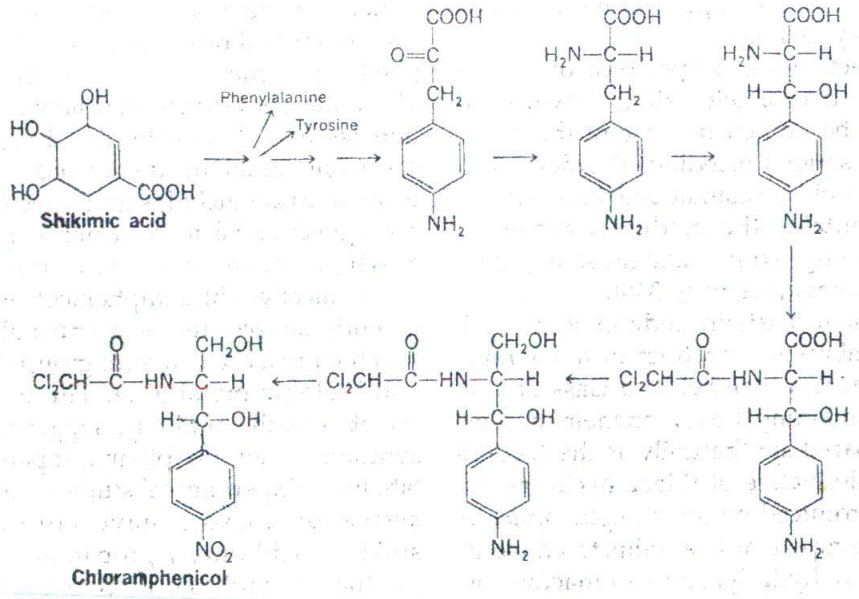


Fig. 12–17. Biosynthesis of chloramphenicol.

Other antibiotics now provide alternate means of controlling many pathogens formerly controlled only by chloramphenicol, which should be used only in serious infections caused by susceptible organisms when other less dangerous antibiotics are ineffective or contraindicated. Third generation cephalosporins are frequently employed alternatives if parenteral administration is no problem. Chloramphenicol may still be the drug of choice for acute typhoid fever, other severe *Salmonella* infections, and rickettsial infections in children between 1 and 8 years of age. Penicillin hypersensitivity and renal insufficiency present considerations that could favor the use of chloramphenicol over ampicillin and tetracycline, respectively. Microbial resistance to chloramphenicol is characterized in many cases by acetylation of the antibiotic. The greatest resistance problem occurs with *Pseudomonas*, but episomal R-factor transfer causes some resistance in other gram-negative bacteria. Multiple resistance to chloramphenicol and the β-lactam antibiotics is known in some gram-positive cocci and some strains of *Haemophilus influenzae*.

Chloramphenicol is stable, but esters of the antibiotic are employed in certain pharmaceutic formulations for solubility purposes. These esters are hydrolyzed in the body to release the physiologically active molecule. The insoluble palmitate ester is used in some oral formulations to avoid the bitter taste of the antibiotic, and the monosodium succinate ester is used for greater water solubility in preparations for intravenous use. Tissue esterases are not as efficient as pancreatic esterases, and approximately one third of parenterally administered chloramphenicol is eliminated renally as the inactive ester.

The usual dose is the equivalent of 50 mg of chloramphenicol per kg of body weight daily in 4 divided oral doses or intravenously in 2 or 3 divided doses. The antibiotic is absorbed readily on oral administration; the usual dose gives blood levels of approximately 10 μg per ml in 2 to 4 hours. The MIC range for most clinically sensitive bacteria is 0.2 to 2.0 μg per ml, but higher doses of the antibiotic are required occasionally for pathogens with an MIC in the 15 to 50 μg per ml range. Chloramphenicol is 60% protein bound in the blood, diffuses readily into other body tissues and fluids, and has a normal biologic half-life of between 2 and 5 hours. Hepatic conjugation with glucuronic acid inactivates approximately 90% of the antibiotic prior to tubular excretion. The balance of the antibiotic is eliminated in free form by glomerular filtration; rapid renal clearance yields urine concentrations of active chloramphenicol that are adequate for therapeutic purposes, but this antibiotic is rarely indicated for urinary tract infections.

PRESCRIPTION PRODUCTS. Chloromycetin®, Mychel®.

Lincomycin and Clindamycin

Lincomycin is produced by *Streptomyces lincolnensis*. It has an amide function in the molecule and may be derived by a combination of amino acid and carbohydrate metabolites. **Clindamycin** (7-chloro-7-deoxylincomycin) is synthetically derived from lincomycin. These antibiotics have primarily gram-positive spectra, including pneumococci, staphylococci, and streptococci, with the exception of *Streptococcus faecalis*; the anaerobic spectra (both gram-negative and gram-positive) are also recognized as distinctive and significant. Clindamycin appears slightly more effective quantitatively than lincomycin; the MICs for most bacteria susceptible to clindamycin are in the 0.01 to 3.1 μg per ml range compared with a range of 0.02 to 6.2 μg per ml for lincomycin.

These antibiotics inhibit protein synthesis by a mechanism closely related to that of chloramphenicol and erythromycin. They all bind to the same site on the 50S subunit of 70S ribosomes. Erythromycin has a greater affinity for the site and thus effectively antagonizes the action of clin-

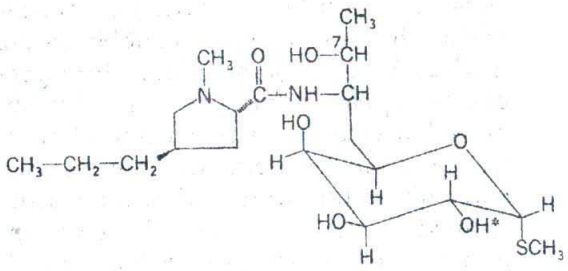
damycin or lincomycin. The absence of aerobic gram-negative spectra for clindamycin and lincomycin may relate to their inability to penetrate the cell walls of these bacteria. Microbial resistance to clindamycin and lincomycin slowly develops, but resistant strains are commonly resistant to multiple antibiotics, especially to erythromycin.

These antibiotics yield effective serum levels readily and exhibit no appreciable protein binding or accumulation, but their significant biologic properties show some variation. Clindamycin is more rapidly and completely absorbed and is more readily eliminated from the body. The usual 500-mg dose of lincomycin gives a peak serum level of 1.8 to 5.3 μg per ml in 4 hours and has a normal half-life in the 4 to 6 hour range. Food does reduce the serum levels that are achieved with lincomycin, and administration on an empty stomach is recommended to avoid this problem; an extended half-life necessitates dosage adjustment in cases of renal disease or hepatic complication. Food does not influence the absorption of clindamycin, and the slight extension of antibiotic half-life with renal dysfunction tends to be insignificant clinically. The usual 300-mg dose of clindamycin gives a peak serum level of 2.6 to 3.6 μg per ml in 1 to 2 hours, and the normal half-life is between 2 and 4 hours.

Both clindamycin and lincomycin can cause severe colitis and pseudomembranous colitis, which may end fatally. It is recommended that their use be reserved for serious infections caused by susceptible anaerobic bacteria or by pneumococci, staphylococci, or streptococci in patients with mitigating considerations, such as penicillin hypersensitivity. Distribution of these antibiotics in bone also favors their use in staphylococcal osteomyelitis.

Lincomycin is available in formulations of the HCl salt, and the usual adult dose is the equivalent of 500 mg of the antibiotic orally, 3 to 4 times a day, 600 mg intramuscularly, 1 or 2 times a day, and 600 mg by intravenous infusion (over a period of not less than 1 hour) every 8 to 12 hours.

PRESCRIPTION PRODUCT. Lincocin®.



Lincomycin

*Esterified (palmitate or phosphate) in some formulations of clindamycin.

Clindamycin is available in formulations of the HCl salt (capsules) and of the HCl salt of the palmitate ester (suspension) for oral administration and of the phosphate ester for parenteral use. The palmitate and phosphate esters are inactive per se, but they are readily hydrolyzed to clindamycin in the body; gradual hydrolysis of the phosphate ester following intramuscular administration gives a flattened, delayed peak serum concentration and a half-life of approximately 5 hours. The usual adult dose is the equivalent of 150 to 450 mg of the antibiotic, orally, 4 times a day and 300 mg, intramuscularly or intravenously, 2 to 4 times daily. The usual pediatric dose is the equivalent of 8 to 25 mg per kg per day divided into 3 or 4 equal doses.

PRESCRIPTION PRODUCT. Cleocin®.

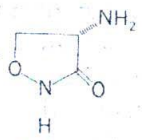
Cycloserine

Cycloserine or D-4-amino-3-isoxazolidinone is probably the simplest metabolite with useful antibiotic activity. It can be produced by cultures of *Streptomyces orchidaceus* or by synthesis. Cycloserine has a fairly broad spectrum of activity, but its therapeutic utility is associated with its inhibitory effect on *Mycobacterium tuberculosis*. This antibiotic inhibits alanine racemase. The inhibitory action precludes the incorporation of D-alanine into the penta-

peptide side chain of the murein component of bacterial cell walls, and this presumably accounts for its antibiotic activity. Cycloserine sometimes causes an increase in the protein content of the cerebrospinal fluid, and this explains in part the CNS effects that may occur when doses exceed 1 g daily. Manifestations of these side effects are usually mental confusion, drowsiness, and coma; cases of psychosis or convulsions are known.

Cycloserine is considered an antibiotic of second choice and is most frequently employed in combination with isoniazid in treating tubercular patients who fail to respond to streptomycin. Cycloserine is readily absorbed following oral administration and is excreted rather rapidly via the kidneys, approximately 50% without metabolic alteration. The usual dose is 250 mg twice a day; the blood level should be monitored and the dosage adjusted to keep the serum level below 30 μg per ml.

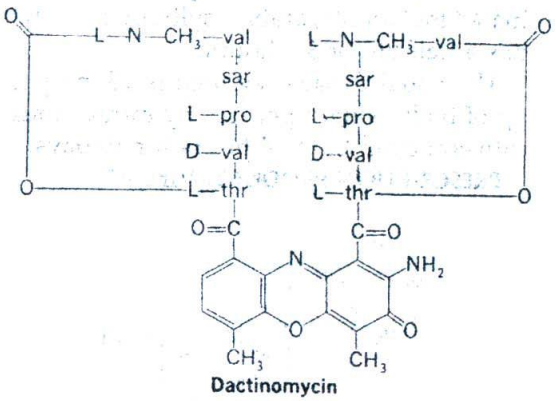
PRESCRIPTION PRODUCT. Seromycin®.



Cycloserine

Dactinomycin

Dactinomycin or actinomycin D is obtained from selected strains of *Streptomyces parvullus* (formerly designated *S. antibioticus*). The molecule contains a phenoxazone chromophore that is linked to 2 cyclic polypeptides. The *N*-methyl amino acids, sarcosine and *N*-methylvaline, are present in the cyclopeptide portions of the antibiotic; this type of amino acid metabolite is uncommon in the plant kingdom. Biosynthetic studies indicate that the phenoxazone portion of the molecule arises from 2 molecules of tryptophan, presumably via the well-established pathway involving 3-hydroxy-anthranilic acid.



Dactinomycin

Dactinomycin is an antineoplastic agent and is used for hospital treatment of Wilms' tumor and several other types of carcinoma and sarcoma. Nausea is common with intravenous administration of dactinomycin, and the best tolerance is obtained in isolated metastases when a perfusion technique can be employed. The drug is available as a lyophilized powder with mannitol. The usual adult dosage regimen is 10 to 15 μg per kg of body weight daily for 5 days by intravenous infusion; therapy is repeated at 4- to 6-week intervals and may involve concurrent administration of other antineoplastic agents.

PRESCRIPTION PRODUCT. Cosmegen®.

Vidarabine

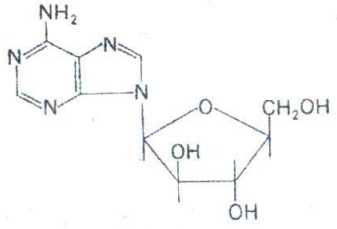
Vidarabine is a purine nucleoside obtained from cultures of a strain of *Streptomyces antibioticus*. It has antiviral activity against *Herpes simplex* virus types 1 and 2. It is indicated for treatment of encephalitis caused by the *Herpes simplex* virus; if treatment is initiated early, the mortality rate can be reduced from 70% to the 28% range. It acts by inhibiting viral DNA synthesis. Vidarabine is teratogenic to laboratory animals, and a safe dose for the human embryo or fetus has not been established.

Vidarabine is administered by slow intravenous infusion. It is rapidly deami-

nated in the body to arabinosylhypoxanthine, a metabolite that has significantly less antiviral activity than does vidarabine. Excretion is renal, primarily as the deaminated metabolite; arabinosylhypoxanthine has a half-life of 3.3 hours.

The usual dosage regimen is 15 mg per kg of body weight per day by intravenous infusion over 12 to 24 hours for 10 days.

PRESCRIPTION PRODUCT. Vira-A®.



Vidarabine

Polypeptide Antibiotics

A fairly large number of **polypeptides** of bacterial origin, which contain both D- and L-amino acids, have antibiotic activity. However, only a few of these metabolites have therapeutic utility. These antibiotics are not absorbed from the intestinal tract, and nephrotoxicity is a potential problem if they are used systemically. Most of the useful peptide antibiotics have a predominantly gram-positive spectrum; exceptions include the strongly basic polymyxins, which are active primarily against gram-negative organisms, and the antineoplastic bleomycin. These peptides have a surfactant property, and the polymyxins exert their effect by interacting with the lipid-rich anionic bacterial cell membrane. However, inhibition of mucopeptide synthesis in cell-wall formation by bacitracin appears to be more significant than membrane disruption for the action of this antibiotic, and inhibition of DNA synthesis is probably the most significant involvement of bleomycin.

The polypeptide antibiotics tend to occur as mixtures of closely related compounds. Components of these mixtures often differ in only 1 or 2 amino acid residues; resolution of such mixtures is not feasible for therapeutic purposes. The use of selected strains of producing organisms controls the composition of commercial mixtures to a degree, and use of microbial assay for quantitation provides a reliable indication of therapeutic response against susceptible organisms.

Polymyxin B is a mixture of antibiotics produced by *Bacillus polymyxa* (Prazmowski) Migula. The mixture contains minimal amounts of the more toxic polymyxins A, C, and D, but the polymyxin B component is actually a mixture of polymyxins B_1 and B_2. Polymyxins B_1 and B_2 contain 10 amino acid residues in common and differ only in a 6-methyloctanoic acid residue in polymyxin B_1 and an isooctanoic acid residue in polymyxin B_2. Both molecules have a cyclopeptidic structure and contain 6 residues of α,γ-diaminobutyric acid. This latter feature gives a strongly basic character to the polymyxin antibiotics.

Polymyxin B is normally employed as the sulfate salt, which must have a potency of not less than 6000 units per mg. *Bordetella bronchiseptica* ATCC No. 4617 is used as the test organism for microbial assay of polymyxin B and the related colistin.

Polymyxin B is not absorbed when administered orally and was formerly employed for control of infections of the intestinal tract caused by *Shigella*, *Pseudomonas aeruginosa*, and *Escherichia coli*. It is used topically in ointments (usually 5000 or 10,000 units per g) and ophthalmic solutions (10,000 units per ml) and parenterally as an alternate antibiotic. Nephro- and neurotoxicities occur fairly frequently when polymyxin B sulfate is used systemically, but it has some limited utility in serious infections of *Pseudomonas aeruginosa* and certain coliform bacilli that do not respond to other antibiotics, such as carbenicillin or gentamicin. Polymyxin is excreted renally and is useful in con-

trolling resistant infections of the urinary tract.

Biopharmaceutic considerations for the polymyxins are complex, and the available data lack significant utility in evaluating therapeutic situations. The antibiotic binds to bacterial and mammalian cell membranes and persists in various body tissues long after it has disappeared from the serum. The in-vitro MIC for most sensitive bacteria is in the lower end of the 0.02 to 4.0 μg per ml range.

The usual adult dose is, by intravenous infusion, 7500 to 12,500 units per kg of body weight in 300 to 500 ml of 5% dextrose injection, 2 times a day; it can also be administered intramuscularly or intrathecally. The dose should be reduced in persons with renal impairment; the high incidence of toxic manifestations in obese patients also suggests need for deviation from a dosage regimen based exclusively on weight.

PRESCRIPTION PRODUCT. Aerosporin®.

Colistin is obtained from cultures of *Bacillus polymyxa* var. *colistinus* and contains primarily colistin A (polymyxin E) with a small amount of colistin B. This antibiotic has essentially the same spectrum and therapeutic utility as polymyxin B. The sulfate salt is used orally and topically, and the sodium salt of a methane sulfonate derivative (colistimethate) is used parenterally.

The colistimethate is inactive, but active compounds are released in the body. Colistimethate is considered the polymyxin formation of choice for intramuscular administration. It is less painful, gives higher serum levels (6 to 25 μg per ml), is poorly bound to cell membranes, and has a shorter half-life (6 to 12 hours). It is claimed to have less systemic toxicity; however, it is not free from nephro- and neurotoxicities, and special caution is necessary in cases of existing renal dysfunction.

The usual dose of colistin is, orally, 5 to 15 mg per kg of body weight daily in 3 divided doses and, intramuscularly or intravenously, 1.25 mg per kg, 2 to 4 times a day.

PRESCRIPTION PRODUCTS. Sulfate salt: Coly-Mycin S®; colistimethate: Coly-Mycin M®.

Bacitracin is produced by an organism of the licheniformis group of *Bacillus subtilis* Cohn & Prazmowski and is a mixture of at least 5 polypeptides. The major component of the mixture is bacitracin A, which is a dodecylpeptide with 5 of the amino acid residues arranged in a cyclic structure. Bacitracin must have a potency of not less than 40 units per mg, unless it is intended for parenteral use; in the latter case, the potency must be not less than 50 units per mg. Bacitracin is assayed microbiologically using *Micrococcus flavus* ATCC No. 10240.

This antibiotic is active against a wide range of gram-positive bacteria. Bacitracin or zinc bacitracin is a component in many ointment formulations for the control of topical infections; ointments usually contain 500 units per g. Parenteral formulations of bacitracin are available, but systemic use is rarely justified owing to problems of nephrotoxicity and to the increasing availability of less toxic alternate antibiotics. Indications for systemic use are restricted to infants with staphylococcal pneumonia and empyema caused by susceptible organisms; it should be used only where adequate laboratory facilities are available and when constant supervision of the patient is possible. It is administered intramuscularly, 2 or 3 times a day, using a dosage regimen based on age and body weight.

Tyrothricin is a mixture of polypeptide antibiotics produced by *Bacillus brevis* Dubos. The peptides of tyrothricin can be grouped into 2 major categories called **gramicidin** and **tyrocidin.** At least 3 polypeptides representing each group are present in commercial tyrothricin. The tyrocidins are basic, usually occur in tyrothricin mixtures as the HCl salts, and constitute the majority of the mixtures. The neutral gramicidins are most active against gram-

positive cocci and usually account for 20 to 25% of tyrothricin mixtures. Gramicidin is soluble in an acetone-ether mixture, and this solvent can be used to dissolve selectively the gramicidin fraction. Gramicidin is a component in formulations for control of topical infections and has replaced tyrothricin for such purposes.

Capreomycin is a mixture of peptides produced by *Streptomyces capreolus*. Capreomycin I is the major component (not less than 90%); capreomycin II accounts for most of the balance of the mixture. Frequent nephrotoxicity is observed with therapeutic use of this antibiotic. Ototoxicity with irreversible auditory impairment and changes in hepatic function are also encountered.

The antibiotic is used as an alternate antitubercular agent in susceptible strains of *Mycobacterium tuberculosis* when other primary agents, such as streptomycin, isoniazid, and rifampin, are ineffective. It is administered intramuscularly as the sulfate salt, and the usual dose is the equivalent of 1 g of the antibiotic daily for 2 to 4 months, then 1 g, 2 or 3 times a week.

PRESCRIPTION PRODUCT. Capastat®.

Vancomycin is a mixture of glucopeptides produced by *Streptomyces orientalis*. The structure of the primary component of the mixture has been determined to be a complex tricyclic aglycone linked glycosidically to glucose and vancosamine moieties. The molecule contains 1 free carboxylic acid residue, 2 chloro-substituted aromatic units, and 7 amide bonds, one of which is a primary amide. It is assayed microbiologically using *Bacillus subtilis* ATCC No. 6633.

Vancomycin has a gram-positive spectrum, and the HCl salt is used primarily as an alternate antibiotic for treating septicemia or endocarditis caused by staphylococci that are resistant to other antibiotics. The antibiotic is not absorbed orally, but oral administration is used for the treatment of staphylococcal enterocolitis and antibiotic-associated pseudomembranous colitis produced by *Clostridium difficile*. Vancomycin acts on bacterial cell walls by inhibiting murein biosynthesis at some step after formation of the nucleotide pentapeptide.

Intramuscular administration is painful and frequently associated with local necrosis; thus, systemic therapy with vancomycin employs intravenous infusion over a period of 20 to 30 minutes. The usual parenteral dose is 500 mg, 4 times a day. This dosage regimen maintains a serum level of 10 μg per ml or more for 1 to 2 hours from the time of injection; most sensitive bacteria have MICs in the 0.2 to 5.0 μg per ml range. The antibiotic has a half-life of approximately 6 hours and is excreted renally. Ototoxicity is the most frequently encountered side effect; the risk is increased with high doses, prolonged therapy, or renal insufficiency.

The usual oral dose is also 500 mg, 4 times a day.

PRESCRIPTION PRODUCT. Vancocin®.

Bleomycin is a mixture of antineoplastic glycopeptides produced by *Streptomyces verticillus*. The mixture can be separated into A and B fractions, and more than a dozen individual components have been reported. Bleomycin A_2 is the major constituent, composing between 55 and 70% of the mixture. Bleomycin B_2 (25 to 32%) is the second major constituent, and material intended for medicinal use must contain not more than 1% of bleomycin B_4. Bleomycin is standardized biologically, and the potency is expressed in units; bleomycin sulfate contains not less than 1.5 units and not more than 2 units of bleomycin per mg.

Bleomycin appears most useful for its palliative effect in some squamous cell carcinomas, but it is useful in lymphomas, testicular carcinomas, and some soft tissue sarcomas. Its low myelosuppressive action may offer clinical advantages. However, pulmonary toxicity frequently necessitates discontinuation of therapy. Bleomycin is preferentially concentrated in tumors, and a bleomycin-technetium 99m complex has

diagnostic potential as a tumor-scanning agent.

Bleomycin is administered parenterally as the sulfate salt. The usual dosage regimen is 0.25 to 0.5 units per kg of body weight once or twice weekly.

PRESCRIPTION PRODUCT. Blenoxane®.

ANTIBIOTICS DERIVED FROM ACETATE METABOLISM

Acetate metabolism normally involves head-to-tail condensation of 2 carbon units or the formation of some type of isoprenoid compound. Both types of metabolism are basic to most protoplasmic systems, as evidenced by the ubiquitous distribution of fatty acids and certain steroids. However, biosynthetically minor deviations at key stages of normal acetate metabolism can result in uncommon metabolites, some of which have antibiotic activity. The therapeutically useful antibiotics derived from acetate metabolism include the tetracyclines, 2 macrolides, a few polyenes, and griseofulvin. These antibiotics are derived from polyketides, and their formation deviates from the fatty acid pathway by a disruption or lack of the normal reduction-dehydration-reduction sequence as the chain elongates. Subsequent metabolic steps yield the characteristic constituents.

Tetracyclines

The **tetracyclines** are a group of actinomycete antibiotics that have a broad spectrum and considerable therapeutic utility (Fig. 12–18). Chlortetracycline was discovered by Duggar in 1948 from *Streptomyces aureofaciens*. *S. rimosus* yielded oxytetracycline in 1950, and tetracycline was found in the antibiotic mixture from *S. aureofaciens* in 1953. The latter observation resulted in patent problems, cross-licensing agreements, a number of legal challenges, and a major governmental investigation. Other minor tetracyclines occur in fermentation mixtures, but only 7-chloro-6-demethyltetracycline (demeclocycline) is currently used in therapy.

Developments in the selection of mutant strains and in manipulations to control chlorination and methylation have proved useful in the fermentative production of various tetracyclines. The presence of aminopterin or other methylation inhibitors in the nutrient mixture favors the formation of 6-demethyltetracyclines, and compounds such as mercaptothiazole aid tetracycline production by inhibiting chlorination. Initially, tetracycline was prepared in commercial quantities by catalytic dehalogenation (hydrogenolysis) of chlortetracycline, but fermentation procedures are currently more advantageous. Doxycycline, methacycline, and minocycline, however, are semisynthetic antibiotics that are prepared by chemical modification of oxytetracycline or tetracycline.

BIOSYNTHESIS OF TETRACYCLINES. Studies with radioactive compounds have confirmed that tetracycline antibiotics originate through acetate-malonate metabolism. Mutant strains of tetracycline-producing organisms have been selected for genetic blocks in the biosynthetic pathway and have been used to clarify a number of the sequential steps.

It is believed that a malonamyl-CoA residue serves as a primer and that 8 malonate units undergo stepwise condensations with the addition of C_2 units and decarboxylation to yield a linear C_{19} polyketide (Fig. 12–19). Carbonyl-methylene condensations yield the tetracyclic pretetramide nucleus. Methylation of the C-6 position of the pretetramide is an early step in the biosynthesis of most tetracyclines, but this step is omitted in the formation of the naturally occurring demethyltetracyclines. Hydroxylation of the C-4 position and dearomatization to yield a 4-keto intermediate appears to precede 7-chlorination. Halogenation must precede introduction of the 4-amino group, which is methylated stepwise. Terminal reactions in the biosynthetic sequence are hydroxylation at C-6

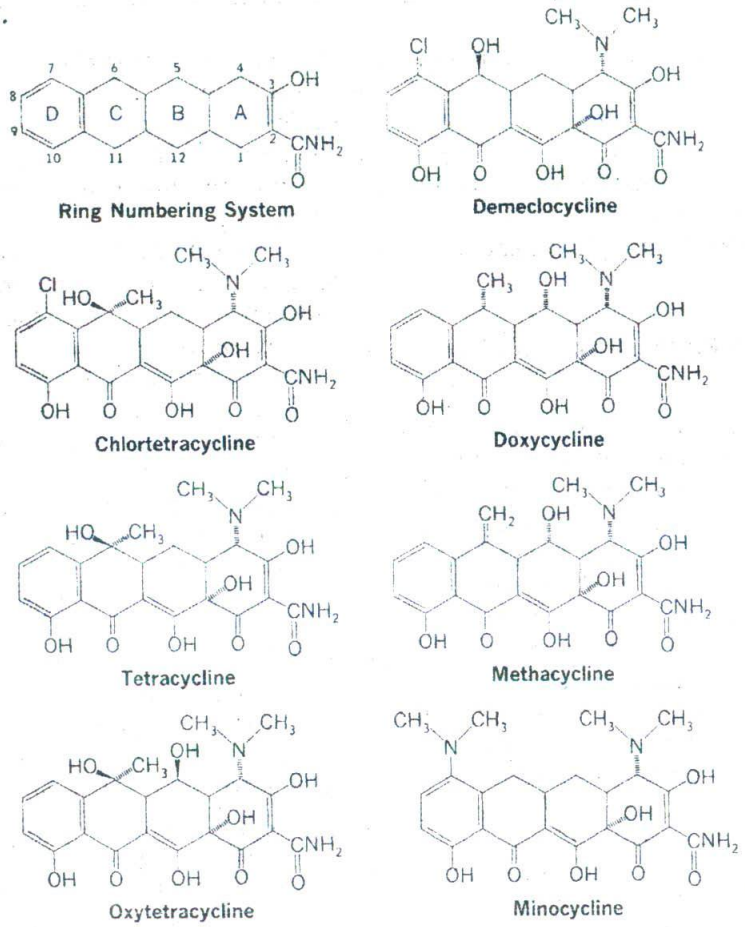


Fig. 12–18. Structures of commercially available tetracyclines.

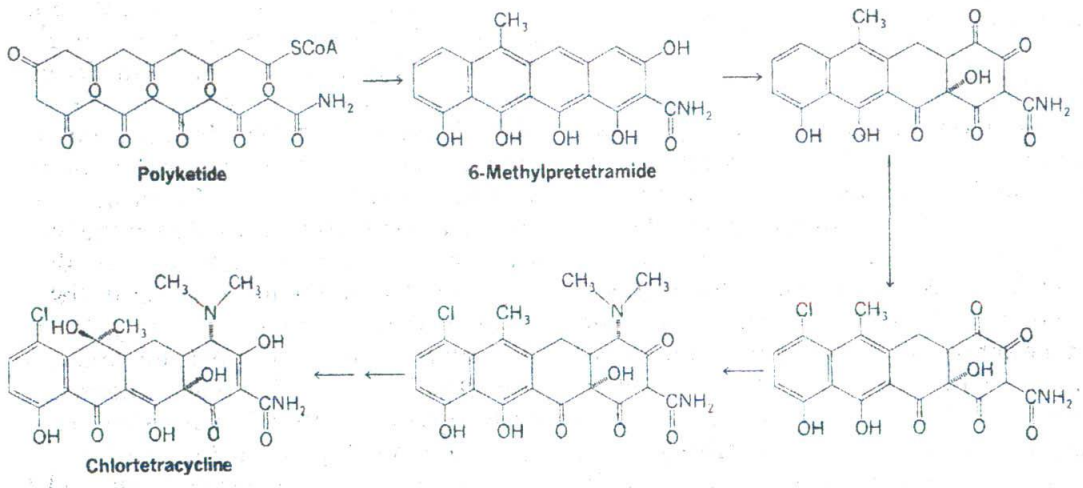


Fig. 12–19. Biosynthesis of chlortetracycline.

and reduction of a double bond in ring B. The 5-hydroxy group in oxytetracycline is probably introduced before the reduction of ring B; it is interesting to note that the presence of a 7-halogen substituent apparently inhibits 5-hydroxylation.

PROPERTIES AND USES OF THE TETRACYCLINES. All tetracyclines are reasonably stable and are absorbed adequately upon oral administration. These amphoteric substances are most stable in acid and least stable in alkali. The tetracycline antibiotics are usually employed as the HCl salts. Chlortetracycline is the least stable of these antibiotics, but it is sufficiently stable for satisfactory oral usage.

Calcium ions in dairy products tend to cause erratic and unsatisfactory absorption of the tetracyclines. Best absorption is obtained when caution is used in scheduling the administration of these antibiotics to avoid interference from heavy metal ions in foods or in such preparations as aluminum hydroxide-containing antacids. Phosphate combinations may be used in tetracycline formulations to reduce the impact of heavy metal ions on absorption. Doxycycline and minocycline are absorbed more readily than the other tetracycline antibiotics, their absorption is influenced to a lesser degree by food and milk, and their slower renal clearance favors prolonged maintenance of blood levels. Doxycycline appears to be the tetracycline of choice when absorption is a problem. The biologic properties of minocycline resemble those of doxycycline, but it is considered a specialty tetracycline at this time. The indication for parenteral use of tetracycline antibiotics is uncommon.

The tetracyclines have a broad spectrum of activity that includes gram-negative and gram-positive bacteria, rickettsia, some of the larger viruses, and some intestinal amoebae. Tetracyclines are often considered the antibiotics of choice for treatment of brucellosis, cholera, relapsing fever, and infections caused by *Chlamydia*, *Mycoplasma*, *Yersinia (Pasteurella)*, and rickettsia. The tetracyclines are effective, alternate-choice antibiotics for treating a large number of other infections. The action spectra for the various tetracyclines are qualitatively comparable, but lower median MICs may favor the use of doxycycline or minocycline in some cases. Normal serum levels on oral regimens are 2 to 4 μg per ml.

The usual serum half-lives of the various tetracyclines are 5 to 6 hours for chlortetracycline, 8 to 9 hours for tetracycline, 9 to 10 hours for oxytetracycline, 12 to 14 hours for demeclocycline and methacycline, and 17 to 19 hours for doxycycline and minocycline. These antibiotics are eliminated by biliary excretion, glomerular filtration, and metabolism. There is extensive enterohepatic recycling of the tetracycline antibiotics, even after parenteral administration. Urinary excretion usually accounts for 20 to 50% of the tetracyclines; the rate of renal clearance is slowest for doxycycline and minocycline. Metabolic degradation of these antibiotics is relatively insignificant, except for chlortetracycline and doxycycline; doxycycline does not accumulate in patients with renal impairment and is the indicated tetracycline in such cases.

Resistance to the tetracyclines developed slowly, but it has become a serious clinical consideration, especially with pneumococci, staphylococci, streptococci, and such gram-negative pathogens as *Escherichia coli* and *Shigella* species. It has been suggested that penicillins, unless specifically contraindicated, should be selected in preference to the tetracyclines for treating susceptible coccal infections. Tetracycline resistance is characterized by an increasing median MIC for strains of various pathogens; the mechanism appears to involve decreased cell permeability to the antibiotics.

Tetracyclines exert their action by inhibiting protein synthesis. The antibiotics interfere with the binding of aminoacyl-tRNA to acceptor sites on the 30S subunit of microbial 70S ribosomes. Tetracyclines

can also attack mammalian 80S ribosomes, but preferential penetration and concentration of these antibiotics in bacterial cells presumably explain the infrequent occurrence of major side effects.

The most frequently encountered adverse effect of tetracycline therapy is alteration of the intestinal flora; this is usually manifested by an overgrowth of *Candida albicans*, but the incidence of tetracycline-associated staphylococcal enterocolitis is increasing. Hypersensitivities may occur; the most serious is a photosensitivity that occurs most often with demeclocycline. The staining of teeth by deposition of tetracyclines in the calcium complex is a basis for selecting alternate antibiotics when treating children during the second dentition period. Hepatotoxicity can also occur, especially in pregnant women, and is usually associated with high blood levels resulting from parenteral administration or renal deficiency. The ability of some tetracyclines to complex with calcium ion can depress plasma prothrombin activity, and patients who are also on anticoagulant drugs may require dosage adjustment.

Chlortetracycline or 7-chlorotetracycline was the first tetracycline antibiotic available for therapeutic purposes. Satisfactory results can be obtained with this antibiotic, and it is still available in formulations for topical use, including ophthalmic purposes. Therapeutic use of other tetracycline antibiotics has replaced its oral and intravenous uses in human medicine, but chlortetracycline is still employed in veterinary medicine.

PRESCRIPTION PRODUCT. Aureomycin®.

Tetracycline is the least expensive and most commonly utilized tetracycline antibiotic. It is available in a large number of formulations of the tetracycline base, HCl salt, and phosphate complex. The usual dosage schedules are based on the equivalence to tetracycline HCl and are 250 to 500 mg, orally, 4 times a day; 250 mg, intramuscularly, once a day or 100 mg, 3 times a day by this route; and 250 to 500 mg, intravenously, 2 times a day. Preparations for topical use are also available.

Low oral doses of tetracycline (250 mg per day) have been used successfully to treat chronic severe cases of acne. The scientific basis for this therapeutic use is unclear. It may be a combination of antibiotic activity reducing slightly the skin population of *Staphylococcus epidermidis* and *Corynebacterium acnes* and of the potential inhibiting effect of tetracycline on bacterial lipase from the latter species. It is believed that acne lesions are related to the irritation caused by free fatty acids in the sebum. Risks of *Candida* superinfection or other toxic responses are minimal with the low dosage regimen, but the prospects for encouraging the selection of resistant strains should preclude the use of tetracycline in trivial cases of acne.

PRESCRIPTION PRODUCTS. Achromycin®, Cycline®, Cyclopar®, Deltamycin®, Nor-Tet®, Panmycin®, Retet®, Robitet®, Sumycin®, Tetra-C®, Tetracap®, Tetracyn®, Tetralan®, Tetram®; phosphate complex: Tetrex®.

Oxytetracycline or 5-hydroxytetracycline is available in various formulations for oral, parenteral, and topical purposes. The insoluble calcium salt is used in oral suspensions, and the oxytetracycline base and the HCl salt are employed, as appropriate, in other dosage forms. The usual dosage schedule is the same as that for tetracycline.

PRESCRIPTION PRODUCTS. Oxymycin®, Terramycin®, Uri-Tet®.

Demeclocycline or 7-chloro-6-demethyltetracycline has greater acid stability than the tetracyclines with a 6-methyl substituent. The better absorption and slower excretion by the body of this tetracycline antibiotic provide blood levels that offer some minor therapeutic advantages. Demeclocycline is used orally as the HCl salt. The usual dose is 600 mg daily in 2 to 4 divided doses.

PRESCRIPTION PRODUCT. Declomycin®.

Doxycycline or 6-deoxy-5-hydroxytetra-

cycline is prepared from oxytetracycline by chemical dehydration and reduction. It is readily absorbed following oral administration, slow excretion gives prolonged blood levels, and no significant accumulation is noted with renal impairment. A suspension of doxycycline base is used orally, and formulations of the water-soluble doxycycline hyclate are available for oral and intravenous administration. The usual oral dosage regimen is the equivalent of 100 mg of the antibiotic 2 times a day for 1 day, then 50 to 100 mg 2 times a day. The usual intravenous schedule is 200 mg on the first day, administered in 1 or 2 infusions, then 100 or 200 mg daily, depending on the severity of infection.

PRESCRIPTION PRODUCTS. Doxy®, Doxychel®, Vibramycin®.

Methacycline is prepared from oxytetracycline by chemical dehydration; it has a methylene function in the 6-position. The utility of methacycline is associated with good oral absorption and a prolonged serum half-life. It is used orally as the HCl salt, and the usual dose is 600 mg daily in 2 or 4 divided doses.

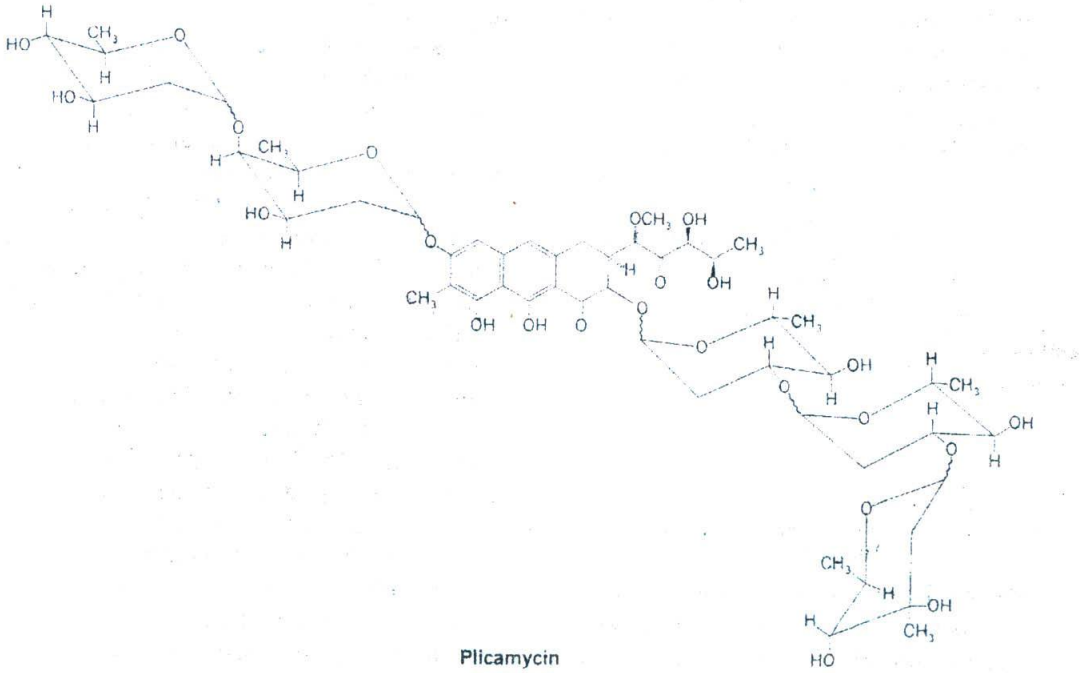
PRESCRIPTION PRODUCT. Rondomycin®.

Minocycline is prepared by reductive methylation of 7-nitro-6-demethyl-6-deoxytetracycline. It is readily absorbed from the intestinal tract, has a slow renal clearance to give prolonged blood levels, and is characterized by lower MICs than other tetracycline antibiotics for some pathogens. Minocycline is especially useful for treating *Neisseria gonorrhoeae* when penicillin is contraindicated and for carrier states of *N. meningitidis*. It is used as the HCl salt, and the usual oral or intravenous regimen involves a loading dose equivalent to 200 mg of the antibiotic, then 100 mg, 2 times a day.

PRESCRIPTION PRODUCT. Minocin®.

Antineoplastic Anthracycline Derivatives

The attention of medical investigators has been attracted to acetate-derived polycyclic metabolites of actinomycetes other than the tetracycline antibiotics. Dauno-



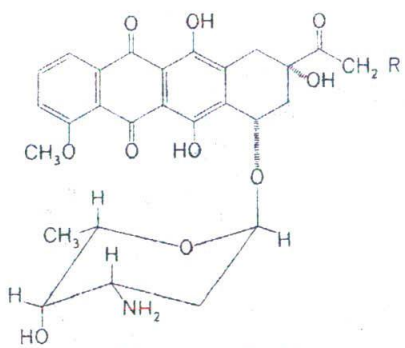
Plicamycin

rubicin, doxorubicin, and plicamycin are 3 such metabolites; they occur as glycosides and have been judged to have utility in treating some neoplastic conditions.

Plicamycin is produced by *Streptomyces agrillaceus* and *S. plicatus*. Plicamycin inhibits DNA replication by forming a stable cross-link between the 2 strands of double-stranded DNA. It is indicated primarily for treatment of disseminated testicular carcinoma when surgery and radiation are contraindicated. Severe toxic reactions restrict administration of plicamycin to selected hospitalized patients, and the therapeutic response tends to be inconsistent. The usual antineoplastic dosage regimen is, by intravenous infusion, 25 to 30 μg per kg of body weight in 1 liter of 5% dextrose injection over a period of 4 to 6 hours once a day for 8 to 10 days or until hematologic or biochemical toxicities require discontinuation. Plicamycin can also be considered for symptomatic control in patients with hypercalcemia and hypercalciuria secondary to a variety of advanced neoplasms.

The antibiotic is unstable, and the lyophilized preparations should be stored at a temperature between 2 and 8° C. Once reconstituted, any unused solution must be discarded.

PRESCRIPTION PRODUCT. Mithracin®.



Doxorubicin: R = OH
Daunorubicin: R = H

Doxorubicin is produced by *Streptomyces peucetius* var. *caesius*. It causes remission in a wide range of solid tumors, but unfortunately, the remission is short-lived in many cases. It shows promise in treatment of some acute leukemias, soft tissue sarcomas, breast cancer, and several types of carcinoma. It is often used as a component in combination chemotherapeutic regimens.

Doxorubicin is rapidly metabolized in the liver (carboxyl reduction) to give an active alcoholic metabolite, adriamycinol. It inhibits DNA-dependent RNA synthesis. Doxorubicin exhibits a high incidence of bone marrow depression and other side effects, such as severe local tissue necrosis and serious irreversible myocardial damage. Complications associated with altered blood coagulation, leg vein thromboses, and pulmonary infarcts are claimed to present fewer problems with doxorubicin than with daunomycin.

Doxorubicin is administered intravenously as the HCl salt. It is excreted in the bile, and enterohepatic recycling gives an extended blood level. Slow renal elimination gives a red coloration to the urine for 1 or 2 days after administration of the drug. The recommended adult dose is 60 to 75 mg per square meter of body surface at 21-day intervals.

PRESCRIPTION PRODUCT. Adriamycin®.

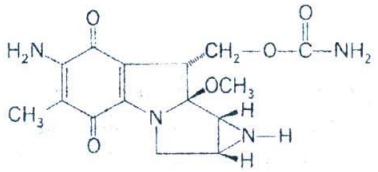
Daunorubicin is produced by *Streptomyces coeruleorubidus*. It is similar to doxorubicin in many of its biologic and chemical properties. Daunorubicin is used to treat acute lymphocytic and nonlymphocytic leukemias, usually as a component of combination chemotherapeutic regimens. It undergoes rapid reductive metabolism in the liver to give the active daunorubicinol.

Daunorubicin is administered intravenously as the HCl salt. The usual adult dose is 30 to 60 mg per square meter of body surface for 2 or 3 days at 3- to 4-week intervals. The pediatric dose is 25 mg per square meter of body surface once a week. The incidence of cardiotoxicity increases significantly for children and adults when the total cumulative doses exceed 300 and 550 mg per square meter of body surface, respectively.

PRESCRIPTION PRODUCT. Cerubidine®.

Mitomycin

Mitomycin C is one of the antineoplastic substances produced by *Streptomyces caespitosus*. It is not significantly more effective than other anticancer agents, and it causes more serious adverse reactions than most. It is considered useful in the treatment of disseminated adenocarcinoma of the stomach or pancreas and is an alternate drug in advanced metastatic conditions of various types that have become resistant to other chemotherapeutic agents. The response rate has been low, as anticipated in such high-risk situations, and remission is usually of short duration.



Mitomycin C

Mitomycin C is inactive per se; the active form is produced metabolically in situ and apparently acts as an alkylating agent to suppress DNA synthesis. Local tissue necrosis may occur, but severe bone marrow depression is the most serious side effect. The usual dosage regimen is, intravenously, 20 mg per square meter of body surface, either as a single dose or as divided doses over 10 days.

PRESCRIPTION PRODUCT. Mutamycin®.

Macrolide Antibiotics

Macrolide antibiotics are characterized by a macrolactone ring that is glycosidically linked to one or more sugars. Biosynthetic studies have established that the macrolactone ring is formed by a condensation of acetate and/or propionate units, apparently via malonyl-CoA and 2-methylmalonyl-CoA. Methyl substituents on the lactone ring appear to be residual from incorporation of propionate units rather than from terminal biologic methylation. The sugar components of these antibiotics are usually deoxysugars, at least one sugar residue is routinely an aminosugar, and both *N*-methyl and *O*-methyl groups of methionine origin are common. Experimental data suggest that these uncommon sugars are derived from glucose. Thus, the macrolides must be considered products of both acetate and carbohydrate metabolism. It is suspected that glycosidation is a terminal reaction in the pathway.

Erythromycin and oleandomycin (Fig. 12–20) are the only macrolide antibacterial agents currently used in therapy. They are produced by actinomycete fermentation, and the large number of asymmetric centers in these antibiotics (19 in erythromycin A) suggests that the potential for chemical modification to develop analogs with improved antibiotic activity is limited and that total chemical synthesis will undoubtedly never become feasible. The macrolactone ring is unstable in gastric acidity, a factor that must be considered when devising pharmaceutic formulations. Enteric coating can be used to deliver the antibiotic to the intestinal tract where it is readily absorbed. The use of insoluble esters, which are hydrolyzed in the intestine and elsewhere in the body, also protects the macrolide antibiotics. This approach offers the additional advantage of masking the bitter taste of these antibiotics in oral suspensions. Erythromycin is absorbed readily from the rectum and may be administered by this route.

Erythromycin and oleandomycin have predominantly gram-positive spectra that resemble those of the penicillins and lincomycin. Erythromycin is slightly more active than oleandomycin, but these antibiotics both have utility, primarily in treating pneumococcal and hemolytic streptococcal infections when penicillins are contraindicated or ineffective. Erythromycin appears to be the antibiotic of choice when treating infections of *Legionella pneumophila*.

Staphylococci frequently become resistant to the macrolides, and this introduces a practical limitation on their therapeutic

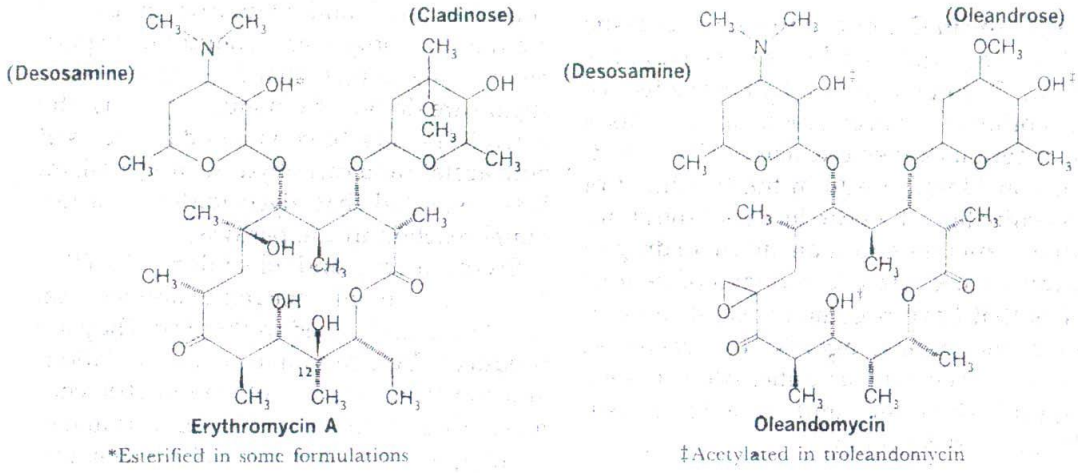


Fig. 12–20. Structures of erythromycin and oleandomycin.

applications to many strains of these pathogens. Several mechanisms of bacterial resistance to macrolides appear to exist. A lack of effective antibiotic penetration of cell membrane explains the relative insensitivity of most gram-negative bacteria to these antibiotics, and similar permeability considerations seem to characterize some resistant strains of normally susceptible species. Destruction of the antibiotics is not a known factor in resistance, but evidence in some cases suggests antibiotic-induced change in the ribosomal structure and an R-factor transfer.

The macrolides exert their action by inhibiting the synthesis of essential proteins. Most of the experimental information has been obtained with erythromycin. It binds to the 50S subunit of microbial 70S ribosomes; it does not inhibit peptide bond formation, but it does block the translocation of the peptidyl-tRNA from the acceptor site to the donor site.

Except for hepatotoxicity in adults taking erythromycin estolate, serious adverse reactions are uncommon with the macrolide antibiotics. They are claimed to be the safest of all currently available antibiotics. Epigastric distress is the most frequently encountered side effect, but this can be reduced by administering the antibiotic with meals. Pseudomembranous colitis has been reported; a temporary hearing loss has also been noted in some older patients, in patients taking unusually high doses of the antibiotic, and in patients with renal insufficiency. The hepatotoxicity can occur with several macrolide formulations, especially erythromycin estolate and triacetyloleanodomycin when used for longer than 10 days. The high incidence of cholestatic jaundice in adults taking erythromycin estolate has prompted most practitioners to restrict the use of this ester to young children.

Erythromycin was isolated in 1952 from cultures of *Streptomyces erythreus*. The commercial product is primarily erythromycin A, but it also contains small amounts of 2 related antibiotics that have been designated erythromycins B and C. Erythromycin B lacks the 12-hydroxyl group that is present in erythromycin A, and erythromycin C has a hydroxyl group rather than a methoxy group in the sugar corresponding to cladinose.

On oral administration erythromycin is absorbed primarily in the lower intestinal tract, and absorption is most efficient for lipid-soluble nonionized forms. Food somewhat retards absorption, and plasma levels vary depending on the form of the drug and on whether it is administered to a fasting patient. The highest levels are ob-

tained with fasting patients and the estolate form of the antibiotic, but most therapeutic needs are met satisfactorily by any of the available formulations and without rigorously excluding the impact of food. There are analytic problems in evalulating effective in-vivo levels of erythromycin formulations, and considerable uncontrolled data can be noted in the literature. When the ester form is employed, a relatively large portion (65 to 80% for erythromycin estolate) of the drug is still unhydrolyzed at the time of peak serum level and thus is inactive.

Normal peak serum levels that have been reported for the usual oral dosage regimens of erythromycin are between 0.3 and 5.0 μg per ml. The antibiotic is distributed with unusual efficiency into most body fluids and tissues, except for the cerebrospinal fluid. Pertinent MICs for erythromycin range from 0.01 to 3.1 μg per ml, with most susceptible gram-positive cocci falling in the lower end of the range; pathogens such as *Haemophilus influenzae* and *Neisseria* species have higher MICs and have more strains that are not susceptible to concentrations ordinarily achieved in therapy.

The normal serum half-life of erythromycin is approximately 1.5 hours, and this is not prolonged greatly in anuria. Some erythromycin is excreted by the kidney (2.5 to 10%), there is extensive enterohepatic recycling, and most of the antibiotic is metabolized prior to elimination. The key involvement of hepatic metabolism may prompt the need for dosage reduction in cases of severe liver disease.

Erythromycin is used orally in various formulations of the free base, the stearate salt, and the ethylsuccinate ester. The lauryl sulfate salt of the propionate ester (estolate) is used in pediatric dosage forms. The soluble glucoheptonate (gluceptate) and lactobionate salts are used for intravenous administration. Ointments (usually 1.0%) are also available for topical purposes. The usual dose, as the equivalent of erythromycin, is 250 mg, orally, 4 times a day for the free base and stearate preparations; 400 mg, orally, 4 times a day for the ethylsuccinate; and 250 mg, intravenously by continuous or intermittent infusion, 4 times a day, for the gluceptate and lactobionate.

PRESCRIPTION PRODUCTS. E-Mycin®, Eryc®, Ery-Tab®, Ilotycin®, Robimycin®, RP-Mycin®; estolate: Ilosone®; gluceptate: Ilotycin®; ethylsuccinate: E.E.S.®, E-Mycin E®, EryPed®, Pediamycin®, Wyamycin E®; lactobionate: Erythrocin®; stearate: Bristamycin®, Eramycin®, Erypar®, Erythrocin®, Ethril®, Pfizer-E®, Wyamycin S®.

Oleandomycin was isolated in 1954 from a strain of *Streptomyces antibioticus*. The insoluble triacetyl ester of oleandomycin (troleandomycin) is available in formulations for oral administration. The usual dose is 250 to 500 mg, orally, 4 times a day. The biologic and chemical properties and the therapeutic considerations for troleandomycin are essentially the same as those for comparable erythromycin formulations. Troleandomycin is an alternate for erythromycin, but it offers no advantage over erythromycin.

PRESCRIPTION PRODUCT. Tao®.

Polyenes

The designation **polyene,** for practical considerations in medicine and pharmacy, refers to a group of amphoteric actinomycete metabolites that are characterized by a series of conjugated double bonds. These metabolites are unsaturated macrolides with macrolactone rings that are considerably larger than those of erythromycin and oleandomycin. They are usually categorized on the basis of the number of conjugated double bonds in the molecules. Nystatin and natamycin, tetraenes, and amphotericin B and candicidin, heptaenes, are the polyenes used in therapy. The polyenes have no antibacterial activity, and their therapeutic utility is related to their antifungal action. The biologic activity of

these antibiotics is determined with various strains of *Saccharomyces cerevisiae*.

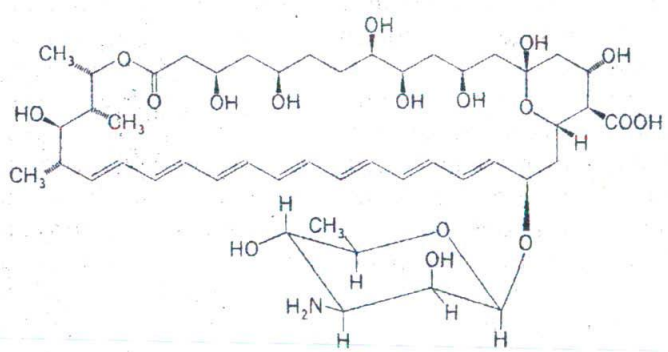
The polyenes are fairly unstable, poorly absorbed from the intestinal tract, and reasonably toxic when administered systemically. They are insoluble, and this property sufficiently protects these antibiotics from inactivation to permit local action in the intestinal tract following oral administration. Limited solubility precludes intramuscular administration of amphotericin B, the only polyene currently recommended for systemic use; therefore, this antibiotic is given by slow intravenous infusion of a formulation that contains sodium deoxycholate to form a colloidal suspension of the polyene.

The polyenes act by destroying the integrity of the cellular membrane of susceptible organisms, and this action may be related to the binding of the polyenes to steroids in the membranes and the formation of aqueous pores. Such a mechanism of action would explain the absence of antibacterial activity because bacterial membranes lack a steroid component. This type of interference with biologic processes may also account for at least one of the adverse reactions observed with systemic use of the polyenes; hemolytic anemia may result directly or indirectly from alteration in the formation or function of cholesterol-containing erythrocyte membranes. The most frequently observed toxicity with systemic use of amphotericin B is nephrotoxicity. Nephrotoxicity with this antibiotic is almost routine, is usually reversible upon cessation of therapy, and must be balanced against the need for control of systemic mycoses in justifying initiation and continuation of therapy in individual cases.

Candida albicans is susceptible to the polyenes, and control of *Candida* overgrowth induced by broad-spectrum antibiotic therapy is a major use of these antifungal agents. Use of polyenes to control *Candida* infections of such origin is justified, but routine incorporation of a polyene in formulations of tetracyclines for prophylactic purposes has been challenged. The challenge is based, in part, on a concern for consequences of any increase in resistance to the polyenes and on a recognition that no alternate antifungal agents are currently available for treatment of systemic candidiasis.

Amphotericin B is produced by *Streptomyces nodosus*, and the commercial product must contain not less than 750 μg of amphotericin B per mg. The less active amphotericin A, a tetraene that is also present in the polyene fraction from cultures of this actinomycete, forms a soluble complex with calcium chloride; this manipulation is used in the commercial preparation of amphotericin B.

Amphotericin B can be used for topical purposes, but its special therapeutic utility is intravenous administration for treatment of potentially life-threatening, dis-



Amphotericin B

seminated mycotic infections, such as blastomycosis, systemic candidiasis, coccidioidomycosis, cryptococcosis, histoplasmosis, and moniliasis. The MICs of susceptible fungi range from 0.03 to 1.0 μg per ml. This antibiotic is slowly eliminated from the body; the plasma half-life is about 24 hours, and the elimination half-life of this strongly protein-bound drug is estimated to be 15 days. Effective blood levels can be maintained with daily administration of a relatively low dose. The usual initial dose is 250 μg per kg of body weight daily, and most regimens call for an increase in the dose every 2 to 4 days for 4 to 8 weeks. Under no circumstances should a total daily dose exceed 1.5 mg per kg. The antibiotic is administered by slow intravenous infusion over a period of 6 hours.

Experimental studies suggest that the action of amphotericin B on cell membranes may have potential use in an interesting type of synergistic combination therapy. The absence of an antifungal spectrum for a number of antibiotics that interfere with protein or RNA synthesis appears to relate to the lack of antibiotic penetration into the fungal cell. A low concentration of amphotericin B in combinations with other selected antibiotics, such as rifampin and tetracycline, seems to facilitate membrane passage of the normally excluded antibiotics; this synergistic action may open new improved therapeutic approaches for treatment of fungal infections.

PRESCRIPTION PRODUCT. Fungizone®.

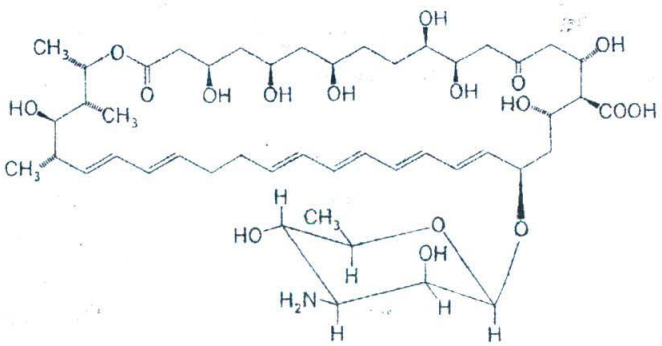
Candicidin is a mixture of heptaenes produced by a strain of *Streptomyces griseus*. It has been formulated as an ointment and a suppository for control of vaginal candidiasis. The recommended dose is 3 mg inserted twice daily for 14 days.

Nystatin is a tetraene produced by *Streptomyces noursei*. The commercial material must contain not less than 4400 units of activity per mg. It is available in formulations for treatment of cutaneous, intestinal, and vaginal infections of *Candida*. MICs range from 1.5 to 6.5 μg per ml. The usual dose is 500,000 to 1 million units, orally, 3 times a day or 100,000 units, intravaginally, 1 or 2 times a day.

PRESCRIPTION PRODUCTS. Candex®, Korostatin®, Mycostatin®, Nilstat®, O–V Statin®.

Natamycin is a tetraene produced by *Streptomyces natalensis*. It is available as a 5% ophthalmic suspension and is used to treat fungal blepharitis, conjunctivitis, and keratitis caused by susceptible organisms, including species of *Aspergillus, Candida, Cephalosporium, Fusarium,* and *Penicillium*. It is the drug of choice for keratitis caused by *Fusarium solani*, an infection occurring in hot, humid climates that frequently leads to blindness.

One drop of a 5% suspension is instilled in the conjunctival sac at intervals of 1 or



Nystatin A_1

2 hours; the frequency of application can be reduced to 1 drop, 6 to 8 times daily after 3 or 4 days, but therapy normally should be continued for 14 to 21 days.

PRESCRIPTION PRODUCT. Natacyn®.

Griseofulvin

Griseofulvin was isolated from cultures of *Penicillium griseofulvum* in 1939, and it was utilized initially in plant pathology for its antifungal activity. Its value in therapeutic control of dermatophytes was not recognized until 1958.

Griseofulvin is also produced by a number of other *Penicillium* species, including *P. janczewski, P. nigrum,* and *P. patulum*. It arises biosynthetically from head-to-tail condensation of 7 acetate units. A polyketide is generally considered the basic precursor (Fig. 12–21), and griseophenone C has been identified as an early intermediate in the pathway. Subsequent methylation and chlorination are believed to precede the oxidative coupling of the benzophenone to the spiran, dehydrogriseofulvin. Presumably, the last step is reduction to yield griseofulvin.

Griseofulvin is stable and only slightly soluble in water. The insolubility of the drug leads to considerable variation in absorption upon oral administration. Formulations of microsize and ultramicrosize griseofulvin are used, and absorption can be facilitated further by administration with a high lipid meal. It is usually employed systemically for control of some dermatophytes belonging to the genera *Epidermophyton, Microsporium,* and *Trichophyton*. Griseofulvin is incorporated preferentially into keratin; this factor explains the unusual oral administration of an antibiotic for dermatomycoses and griseofulvin's lack of therapeutic efficacy in deep mycoses. Sensitive fungi exhibit an unusually narrow range of MICs (0.22 to 0.44 μg per ml).

Further studies are necessary to establish conclusively the means by which griseofulvin exerts its antifungal action. It appears to inhibit fungal cell mitosis by causing disruption of the mitotic spindle structure.

Griseofulvin is administered orally, and the usual dose of the microsize drug is 250 mg, 2 times a day. The ultramicrosize drug achieves about 1.5 times the effect of the microsize form on a unit weight basis. A 3- to 4-week treatment period is adequate for many conditions, but continued therapy for 6 to 12 months is necessary in some cases (e.g., infections of the fingernails or toenails). Griseofulvin is generally free of serious side effects; the most frequently encountered adverse reactions involve hypersensitivity, including occasional photosensitive reactions.

PRESCRIPTION PRODUCTS. Fulvicin®, Grifulvin®, Grisactin®, Gris-PEG®.

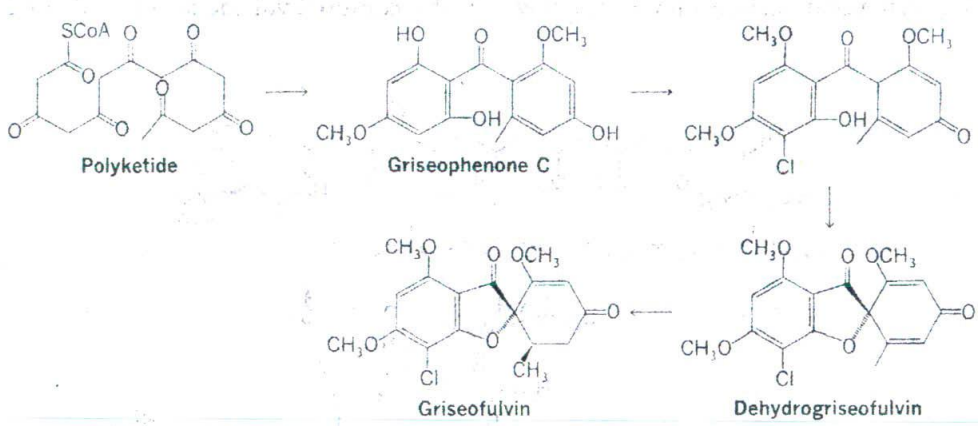


Fig. 12–21. Biosynthesis of griseofulvin.

Rifampin

Rifampin is a semisynthetic antibiotic that is derived from rifamycin B, a metabolite of *Streptomyces mediterranei*. Rifampin has a distinctive macrocyclic lactam structure. The antibiotic inhibits DNA-dependent RNA-polymerase activity in susceptible cells. It has a good gram-positive and a moderate gram-negative spectrum, but its clinical significance is based primarily on the sensitivity of *Mycobacterium tuberculosis* to the antibiotic. It is recommended for treatment of pulmonary tuberculosis; it should be used in combination with at least one other antitubercular agent to avoid selective development of resistant strains of the tubercle bacillus. Rifampin is also useful in the treatment of asymptomatic carriers of *Neisseria meningitidis* when the risk of meningococcal meningitis is high.

Rifampin, in contrast to the naturally occurring rifamycins, is absorbed adequately on oral administration. Peak serum levels with usual dosage regimens are 4 to 32 μg per ml in 2 to 4 hours; the MICs of sensitive strains of *M. tuberculosis* have been reported to range between 0.006 and 0.5 μg per ml. The biologic half-life of rifampin is approximately 3 hours. Urinary excretion may account for elimination of up to 15% of the drug, but biliary excretion is the major pathway. Rifampin is deacetylated in the liver to give an antimicrobially active metabolite, and most of the antibiotic is in the deacetyl form when it is ultimately eliminated in the feces. Enterohepatic recycling of rifampin occurs, but the deacetyl form is not reabsorbed after biliary excretion.

Rifampin is relatively free of toxicity. The most serious adverse reactions involve liver dysfunction, and the increased risk of toxicity in persons with liver damage, such as chronic alcoholics, may preclude the use of this antibiotic. Unfortunately, there is a high incidence of tuberculosis among alcoholics.

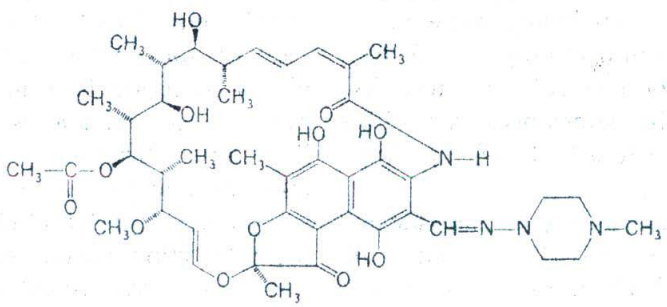
Rifampin is administered orally, and the usual dose is 600 mg, once a day. It should be taken 1 hour before or 2 hours after meals to avoid food interference with absorption. Patients should be advised that the antibiotic may color stools, urine, saliva, sweat, or tears a red-orange.

PRESCRIPTION PRODUCTS. Rifadin®, Rimactane®.

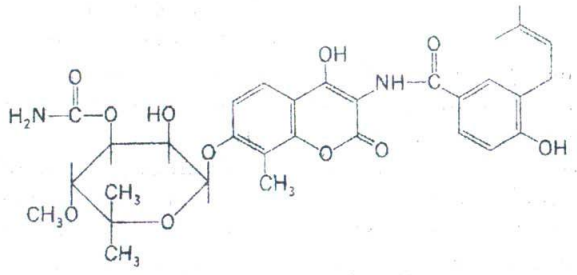
Novobiocin

Novobiocin is produced by *Streptomyces niveus* and *S. spheroides*. The structure of novobiocin suggests an unusual biosynthetic origin for this antibiotic; it appears to involve moieties derived from amino acid, acetate, and carbohydrate metabolic pathways.

The activity spectrum for novobiocin is predominantly gram-positive. Staphylococci tend to be unusually sensitive to this antibiotic (MIC range of 0.1 to 2.0 μg per



Rifampin



ml, but resistance develops rapidly), and it has been used as an alternate means for controlling penicillin-resistant staphylococci. It is also useful in controlling some strains of *Proteus vulgaris*. However, novobiocin has a high incidence of adverse reactions (hypersensitivity, hepatic dysfunction, and blood dyscrasias), and it is recommended only for use in serious infections when other less toxic drugs are ineffective or contraindicated. The penicillinase-resistant penicillins and other available antibiotics have obviated much of the former need for novobiocin; a number of authorities feel that its use can no longer be justified.

It is absorbed rapidly following oral administration. Peak serum levels of 10 to 20 μg per ml are achieved in 2 to 4 hours, and the normal plasma half-life is between 2 to 4 hours. Renal elimination is insignificant (approximately 3%); excretion is primarily biliary, and there is some recycling. The hepatic toxicity of novobiocin may be explained, in part, by its interference with glucuronyl transferase and the consequent disruption in normal biliary excretion of various glucuronide conjugates. Strong protein binding of novobiocin and displacement of other substances from binding sites also create a high risk for drug-drug interactions.

Novobiocin is available as the calcium and sodium salts for oral administration. The usual dose is 250 mg every 6 hours.

PRESCRIPTION PRODUCT. Albamycin®.

ANTIBIOTICS DERIVED FROM CARBOHYDRATE METABOLISM

Carbohydrates provide the basic metabolic substrate for the formation of essentially all microbial products, but this category of antibiotic substances is restricted to compounds that are derived directly from carbohydrate precursors and retain a recognizable carbohydrate character. The therapeutically useful antibiotics derived from **carbohydrate metabolism** include amikacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, spectinomycin, streptomycin, and tobramycin.

The chemical and biologic properties of these antibiotics are similar. Common chemical properties include water solubility, a strongly basic character, and stability. The antibiotic molecules, except for spectinomycin, routinely have 2 to 3 uncommon sugars linked glycosidically to an amino-substituted cyclohexanyl aglycone. The designation, aminoglycoside antibiotics, is used as a generic term for these compounds. Normally, antibiotic mixtures of closely related molecules are obtained by fermentation, and resolution of the individual components is infeasible and unnecessary for therapeutic purposes. Amikacin and netilmicin are different because they are semisynthetic materials produced from kanamycin A and sisomicin, respectively. Spectinomycin is technically an aminocyclitol derivative rather than an aminoglycoside, but a number of its key

properties are similar to those of the aminoglycoside antibiotics.

The aminoglycoside antibiotics have a wide spectrum of activity, including many gram-negative and gram-positive bacteria. These antibiotics are not absorbed following oral administration, and their systemic use is limited by nephro- and ototoxicities. The consequences of ototoxicity may be unusually serious. These antibiotics tend to damage both the auditory and vestibular branches of the 8th cranial nerve. Vestibular involvement is observed more frequently, especially with gentamicin and streptomycin. Symptoms include nausea, vertigo, and even vomiting, but recovery is usually complete when therapy is discontinued. Damage of the auditory branch results in irreversible loss of hearing; auditory toxicity appears to be more common with amikacin, kanamycin, neomycin and netilmicin.

The aminoglycoside antibiotics act on the 30S subunit of 70S ribosomal systems to induce specific misreading of the genetic codon and to inhibit the formation of essential bacterial proteins by interfering with the initiation complex between RNA and the 30S subunit or by disrupting translocation. The misreading of coded information yields proteins that lack the distinctive physiologic function of normal microbial proteins, but blockage of protein synthesis is believed to be the more therapeutically important mechanism of action.

Emergence of resistant strains, especially of gram-negative bacilli, staphylococci, and mycobacteria, is becoming an increasing problem with the aminoglycoside antibiotics and has contributed, in part, to a decreasing therapeutic utility, especially for kanamycin, neomycin, and streptomycin. Known mechanisms of resistance include chromosomal involvement (alteration of the reactive site on the 30S ribosomal subunit), plasmid transfer of extrachromosomal R-factors, and exclusion of the antibiotic from the bacterial cell. The greatest clinical problems are associated with resistance caused by R-factor transfer; enzymatic inactivation of one or more of the aminoglycosides is accomplished by acetylation, adenylation, or phosphorylation. Cross-resistance among the various aminoglycoside antibiotics is often complete, but no or only partial cross-resistance is observed with some bacteria, depending on the nature of the metabolic inactivation that is involved. For example, if streptomycin is inactivated by phosphorylation of the 3-hydroxyl function of 2-deoxy-*N*-methylglucosamine, cross-resistance can be expected with kanamycin and paromomycin; if the same position is adenylated, cross-resistance occurs with spectinomycin.

The need for therapeutic control of gram-negative organisms and mycobacteria contributes to the therapeutic importance of the aminoglycoside antibiotics. However, the high incidence of resistance and the considerable variation that has been noted in some biologic properties of the antibiotics require efforts greater than normal with the administration of these drugs to ensure effective utilization. Strain sensitivity should be determined routinely, and blood levels should be monitored periodically. A serum plasma level between 4 and 16 μg per ml is usually desired for most aminoglycoside antibiotics and most pathogens, and dosage regimens should be adjusted individually as needed. Renal conditions have an unusually profound influence on their excretion, which is predominantly by glomerular filtration. Renal impairment can increase the biologic half-life from 2 or 3 hours to several days; in such cases, drastic adjustments in the dosage regimen must be made to avoid prolonged high serum levels and the associated increased risks of toxic reactions.

The aminoglycoside antibiotics are indicated only for the treatment of serious infections when less toxic antibiotics are ineffective or contraindicated.

Streptomycin

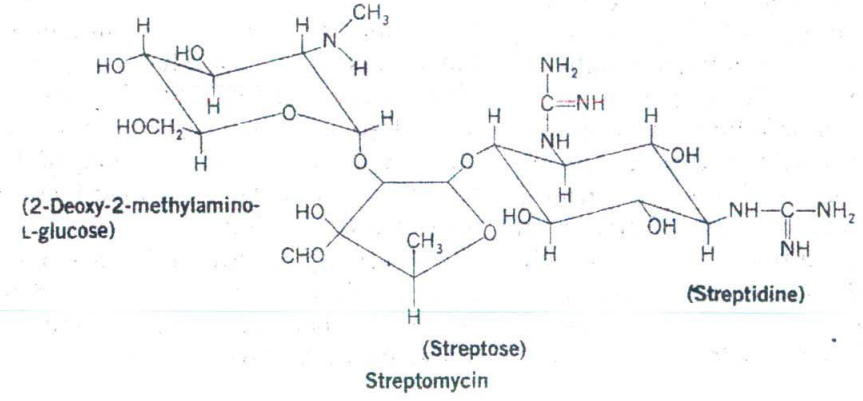
Recognition of the therapeutic potential of penicillin stimulated an intensive search for other antibiotic substances. A special objective of these efforts was the discovery of antibiotics antagonistic to gram-negative bacteria. **Streptomycin** was isolated from a strain of *Streptomyces griseus* by Waksman and coworkers in 1944 after they had noted the in-vitro inhibitory effect of metabolites of this species on gram-negative bacteria.

Because streptomycin was the first aminoglycoside antibiotic to be discovered, studies of its origin and properties provide the basis for much of the current knowledge about this group of antibiotics. Components of streptomycin include streptidine and the disaccharide, streptobiosamine, which contains the sugar residues, 2-deoxy-2-methylamino-L-glucose and streptose. Biosynthetic studies have shown that all 3 of these components are derived from D-glucose. No definitive information is available on the linking of the 3 components, but it is probably a terminal phase of the biosynthetic sequence. Detailed knowledge on the formation of individual moieties of aminoglycoside antibiotics is limited, but a general indication of the metabolic relationships of glucose to the various moieties can be gained from the biosynthetic origins of the streptomycin components (Fig. 12–22).

The nephro- and ototoxicities common to the aminoglycoside antibiotics are encountered with the systemic use of streptomycin. The high incidence of hypersensitivity of streptomycin, even on topical contact, is less serious. Hypersensitivity is not a major adverse response to aminoglycoside antibiotics as a group, and it is probably related in this instance to hapten formation involving the formyl group of the streptose unit in streptomycin.

The potential toxicity associated with systemic use of streptomycin is such that the antibiotic is considered for therapeutic use only when satisfactory alternatives are unavailable. *Mycobacterium tuberculosis* is refractive to most antibiotic therapy, and tuberculosis is the major condition requiring systemic administration for which streptomycin is a first-choice antibiotic. In treatment of tuberculosis, streptomycin is normally combined with ethambutol and isoniazid to achieve the best results. Justification is lacking for earlier claims that dihydrostreptomycin, which can be prepared fermentatively with *S. humidus* or chemically from streptomycin by catalytic reduction of the formyl substituent on the streptose unit, could be used with streptomycin to reduce toxicity. The incidence of serious auditory impairment is now recognized to be greater with dihydrostreptomycin than with streptomycin.

Streptomycin has some value in controlling *Yersinia pestis* (plague) and *Francisella tularensis* (tularemia); in such cases, it is usually combined with a sulfonamide. Combined streptomycin-penicillin and



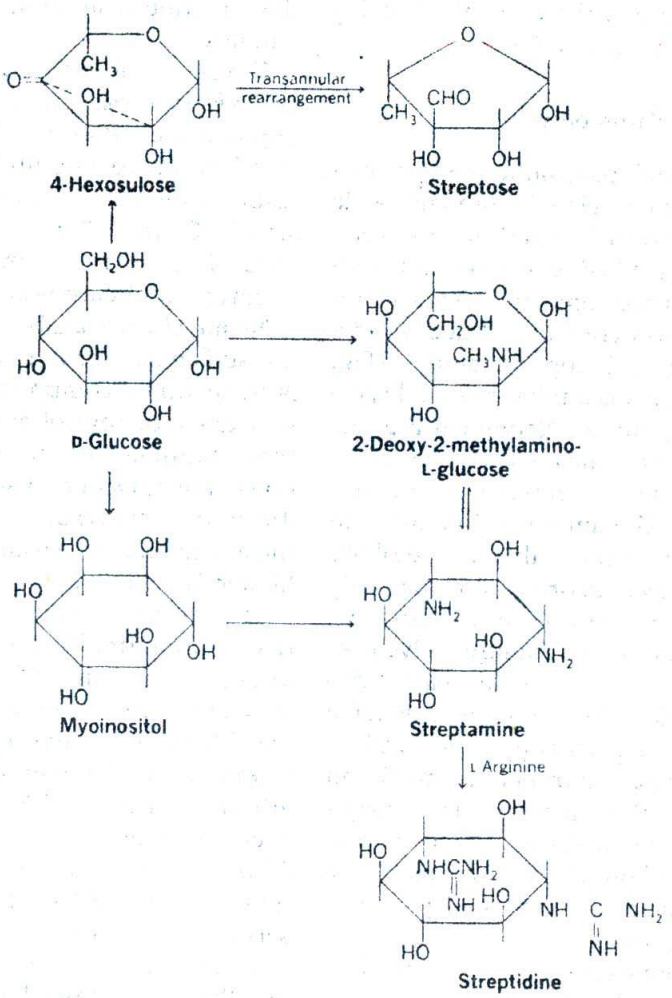


Fig. 12–22. Biosynthesis of components of streptomycin.

streptomycin-tetracycline therapeutic approaches are sometimes indicated in bacterial endocarditis and brucellosis, respectively.

The antimicrobial activity of streptomycin and other aminoglycoside antibiotics is significantly greater in slightly alkaline conditions than in acidic environments, a factor that can be exploited beneficially in urinary tract infections. The MIC of streptomycin for *M. tuberculosis* is approximately 0.5 μg per ml; many sensitive gram-negative bacteria have MICs in the 2 to 4 μg per ml range. A 1-g intramuscular dose usually gives peak serum levels of 25 to 50 μg per ml in 1 to 2 hours; the normal half-life is 2.5 to 3 hours. Peak serum levels are not as reliable an indicator of potential ototoxicity with streptomycin as are the 24-hour levels following daily injections; toxicity risks increase with 24-hour levels exceeding 5 μg per ml. Because serum levels are prolonged in patients with kidney impairment, peak serum levels should not exceed 20 to 25 μg per ml in such cases.

Streptomycin is available in formulations of its sulfate salt. The biologic efficacy of streptomycin and preparations of this antibiotic can be measured with *Klebsiella pneumoniae* ATCC No. 10031. The usual in-

tramuscular dose is the equivalent of 1 g of streptomycin, once a day.

Neomycin and Paromomycin

Neomycin and paromomycin are mixtures of chemically related aminoglycoside antibiotics that were isolated, respectively, from *Streptomyces fradiae* in 1949 and *S. rimosus* var. *paromomycinus* in 1959. The antibiotic molecules contain a 2-deoxystreptamine unit and 3 sugar residues (Fig. 12–23). Neomycin is a mixture of at least 3 antibiotic compounds. Neomycin B is the main component of the mixture. Neomycin C differs from neomycin B only in the stereochemistry of the aminomethyl group in the aminosugar that is linked to the ribose residue. Neomycin A or neamine has only a single sugar residue (neosamine C) linked to the deoxystreptamine aglycone.

Paromomycin consists of at least 2 different antibiotics. These compounds have been designated paromomycins I and II, and they are analogs of neomycins B and C, respectively. Paromomycin I is the major component in the mixture, and the paromomycins differ from the neomycins by the replacement of one amino group with a hydroxyl function.

These antibiotics are stable, not absorbed following oral administration, and have the activity spectrum that is generally characteristic of the aminoglycoside antibiotics. Neomycin or other aminoglycoside antibiotics can be taken orally to control intestinal infections by susceptible organisms or for pre- or postoperative reduction of the intestinal flora. These antibiotics reduce the population of ammonia-forming bacteria in the intestinal tract, and they are used as effective adjunctive therapy in hepatic coma.

Oral administration of aminoglycoside antibiotics favors the emergence of resistant strains. Anaerobic bacteria, the major component of the bowel flora, are not sensitive to these antibiotics. Many authorities recommend restriction of oral administration to serious conditions and high risk situations.

The MICs for such intestinal pathogens as *Escherichia coli* and *Shigella* species are approximately 8 μg per ml. Paromomycin also has therapeutic utility in treating intestinal amebiasis. *Staphylococcus epidermidis* ATCC No. 12228 can be used as a microbial test organism for evaluating neomycin and paromomycin.

Neomycin is available in formulations of the sulfate salt for oral and topical use. It is frequently a component (0.35%) in formulations for control of topical infections; these formulations are usually combinations of neomycin and such agents as bacitracin or polymyxin B, which discourage the emergence of resistant strains. This antibiotic is used orally for preoperative reduction of the intestinal flora and for control of intestinal infections. The usual dosage for intestinal infections is the equivalent of 8.75 mg of neomycin per kg of body weight, every 6 hours, for 2 to 3 days. Preoperative use normally involves oral administration of 700 mg of neomycin every hour for 4 doses, then 700 mg every 4 hours for the balance of 24 hours. Intramuscular administration of neomycin is reserved for hospitalized patients with infections by susceptible pathogens for which no other antimicrobial agent is effective.

PRESCRIPTION PRODUCTS. Mycifradin®, Neobiotic®.

Paromomycin is available in formulations of the sulfate salt for oral administration. This antibiotic should be taken with meals. The usual dosage regimen is the equivalent of 25 to 35 mg of paromomycin per kg of body weight daily, taken in 3 divided doses for 5 to 10 days.

PRESCRIPTION PRODUCT. Humatin®.

Kanamycin

Kanamycin was isolated from *Streptomyces kanamyceticus* in 1957 and is a mixture of at least 3 aminoglycoside antibiotics. These antibiotics contain 2 aminosugars that are linked individually to a 2-deoxy-

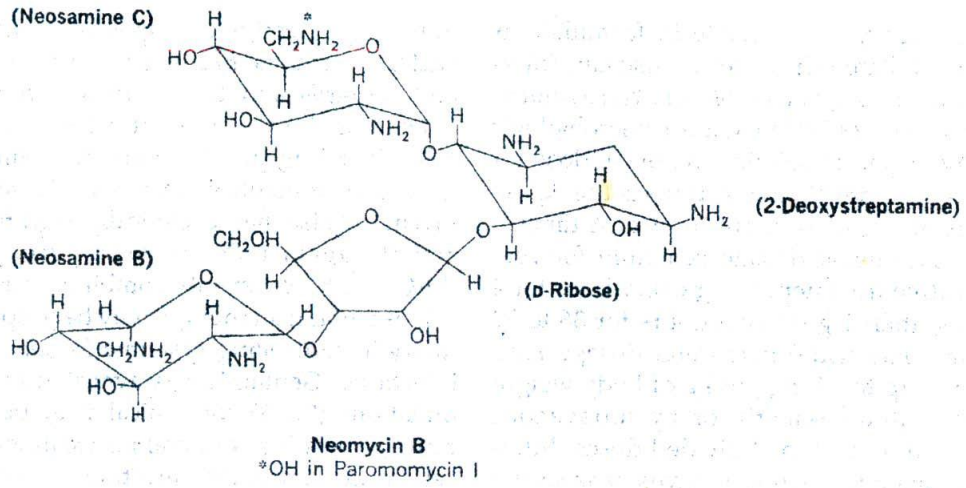


Fig. 12–23. Structures of neomycin B and paromomycin I.

streptamine aglycone. Kanamycin A is the major component of the mixture (Fig. 12–24).

Kanamycin has an activity spectrum that is comparable to the other aminoglycoside antibiotics. It is used orally for control of infections and for preoperative treatment. The coliform bacteria are sensitive to kanamycin, and *Proteus* species are usually more susceptible to it than to the older aminoglycoside antibiotics. MICs for sensitive gram-negative bacilli usually fall in the 4 to 8 μg per ml range. Kanamycin can be used parenterally for treatment of serious gram-negative infections when susceptible strains are involved. Emerging resistance has become a problem, and amikacin, gentamicin, and tobramycin have replaced kanamycin for systemic treatment of most gram-negative pathogens.

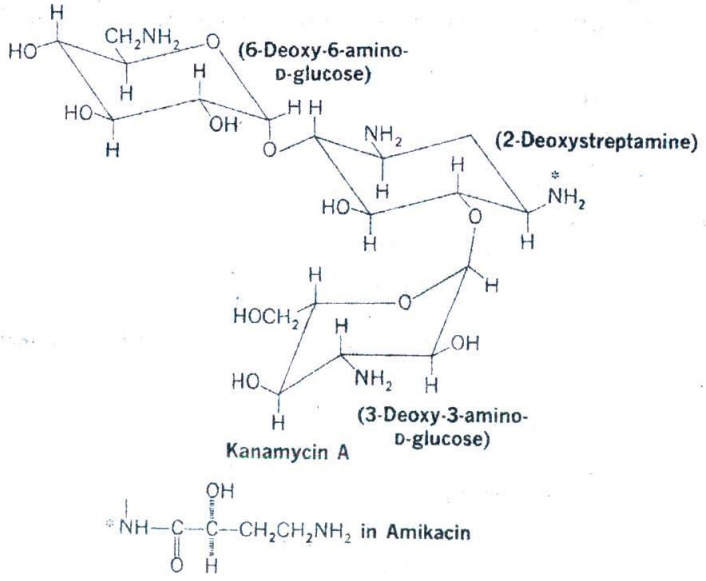


Fig. 12–24. Structures of kanamycin A and amikacin.

Kanamycin is available in formulations of the sulfate salt for intramuscular, intravenous, and oral use. *Staphylococcus aureus* ATCC No. 29737 is used for microbiologic assay of this antibiotic. The usual dose for control of intestinal infections is the equivalent of 1 g of kanamycin, 3 or 4 times a day. The usual dosage schedule for preoperative treatment is 1 g every hour for 4 doses, then 1 g every 6 hours for 36 to 72 hours. The usual parenteral dosage regimen is up to 15 mg per kg of body weight daily, intramuscularly or by intravenous infusion, in 2, 3, or 4 divided doses. Intramuscular administration gives peak serum levels of approximately 20 μg per ml in 1 to 2 hours; the normal half-life is between 2 and 4 hours.

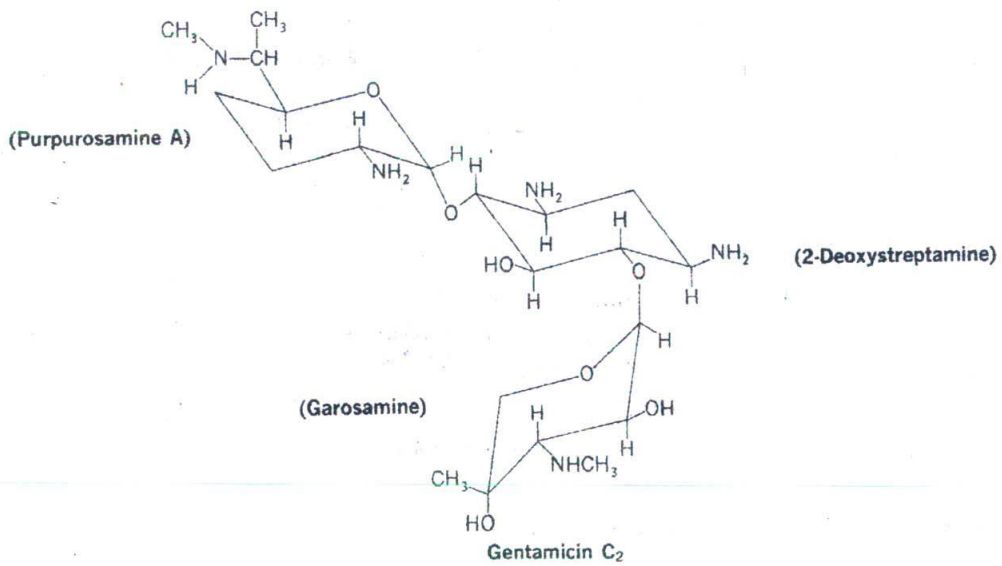
PRESCRIPTION PRODUCTS. Kantrex®, Klebcil®.

Gentamicin

Gentamicin is produced by *Micromonospora purpurea*, an actinomycete. The antibiotic mixture used in medicine consists primarily of gentamicin C_1, C_{1A}, and C_2. Gentamicin C_1 is the major component (approximately 60%). These antibiotic substances contain 2 aminosugar residues and a 2-deoxystreptamine unit. Gentamicin is inhibitory to pathogenic species of enterobacteria such as *Enterobacter*, *Escherichia*, and *Klebsiella* and to *Proteus* and *Serratia* species in lower concentrations (usual MIC, 1 to 2 μg per ml) than other aminoglycoside antibiotics, exclusive of tobramycin. It also has a clinically significant activity against *Pseudomonas aeruginosa* (MIC 2 to 8 μg per ml); combined carbenicillin-gentamicin therapy may have special utility in controlling systemic *Pseudomonas* infections. Gentamicin is available in formulations (0.1%) for topical use, but its principal use is parenteral for treatment of serious gram-negative infections caused by sensitive organisms.

Resistance to gentamicin occurs, but cross-resistance with other aminoglycoside antibiotics is absent in many clinical situations; the lack of cross-resistance is presumably related to R-factor-induced inactivation involving specific chemical sites that are not found in the gentamicin molecule (e.g., inactivation by adenylation or esterification of 3-hydroxyl function of a glucosamine moiety).

Gentamicin is rapidly absorbed on intramuscular administration and is readily distributed into various body tissues. Peak serum levels are often achieved in less than



1 hour, and the normal serum half-life is approximately 2 hours. It has been observed that dosage regimens based arbitrarily on mg per kg of body weight result in widely varying plasma levels that may be ineffectively low or dangerously high; for this reason, monitoring of plasma levels and individualization of dosage regimens are highly recommended with this antibiotic. The risk of ototoxicity increases greatly with prolonged serum levels greater than 10 to 12 μg per ml, and trough levels above 2 μg per ml should be avoided.

Gentamicin is available as the sulfate salt, and the usual adult dose is the equivalent of 1 mg of gentamicin per kg of body weight, intramuscularly or intravenously, 3 times a day.

PRESCRIPTION PRODUCTS. Apogen®, Bristagen®, Garamycin®, Jenamicin®.

Tobramycin

Tobramycin or nebramycin factor 6 is the single-component antibiotic that is separated from the nebramycin complex produced by *Streptomyces tenebrarius*. This antibiotic substance contains 2 aminosugar residues and a 2-deoxystreptamine unit; it is structurally related to kanamycin B, differing only in the absence of the 3-hydroxyl function in the kanosamine residue.

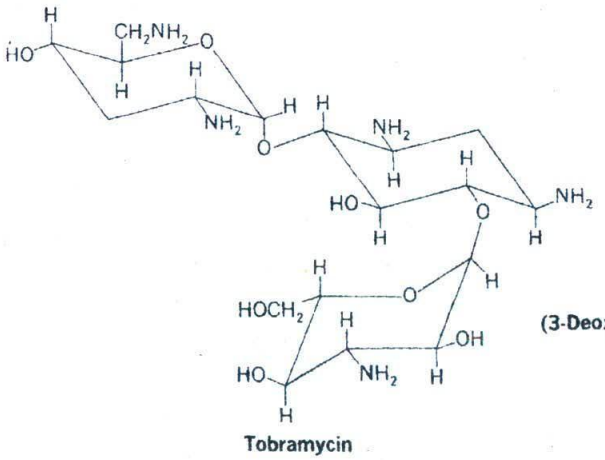
Tobramycin was approved in mid-1975 for general medical use. It has biologic properties and clinical indications that are similar to those for gentamicin. Tobramycin may give slightly lower tissue levels than gentamicin, and *Proteus vulgaris* and *Pseudomonas* species are more sensitive in vitro to tobramycin; however, these differences appear to lack significance in clinical situations.

Tobramycin is available as the sulfate salt, and the usual adult dose is the equivalent of 1 mg of tobramycin per kg of body weight, intramuscularly or intravenously, 3 times a day.

PRESCRIPTION PRODUCT. Nebcin®.

Amikacin

Amikacin is a semisynthetic aminoglycoside antibiotic derived from kanamycin A by acylation of the 1-amino group of the deoxystreptamine moiety to add an L-(−)-4-amino-2-hydroxybutyryl substituent (see Fig. 12–24). The terminal amino group in this substituent is apparently essential for activity; amikacin is active against many strains of pathogens that inactivate gentamicin, tobramycin, and other aminoglycoside antibiotics by enzymatic *N*-acetylation. Pathogens resistant to amikacin are invariably resistant to other known aminoglycoside antibiotics, a consideration that has prompted some authorities to recommend its conservative or restricted use.



Amikacin is readily absorbed following intramuscular administration, and its normal serum half-life is approximately 2 hours. Risks of ototoxicity suggest that the peak serum level should not exceed 35 μg per ml and that trough levels should not exceed 10 μg per ml. A serum level of 8 μg per ml is adequate to exceed the MICs of 90% of the strains of *Escherichia coli, Enterobacter, Klebsiella,* and *Proteus.* Levels of 25 μg per ml are required to reach 90% of the MICs for strains of *Pseudomonas, Serratia,* and *Staphylococcus aureus.*

Amikacin is available as the sulfate salt. The usual dosage regimen for patients with normal renal function is 15 mg per kg of body weight daily, intramuscularly or by intravenous infusion, in 2 or 3 divided doses for 7 to 10 days. Use of ideal body weight is recommended in dosage calculation, and heavier patients should not receive more than 1.5 g per day.

PRESCRIPTION PRODUCT. Amikin®.

Netilmicin

Netilmicin or N-ethylsisomicin is a semisynthetic aminoglycoside antibiotic derived from sisomicin, a product of *Micromonospora inyoensis.* It resembles gentamicin C_{1A} chemically. It is effective against a number of the gram-negative pathogens that are resistant to amikacin, gentamicin, and tobramycin.

Netilmicin is rapidly distributed in body organs and tissues following parenteral administration. The normal serum half-life is approximately 2 hours. Ototoxicity considerations suggest that the peak serum level should not exceed 16 μg per ml and that the trough serum level should not exceed 4 μg per ml. Nephrotoxicity appears to be less of a consideration than with other aminoglycoside antibiotics, and urine concentrations of up to 800 μg per ml are attained. It is useful in treating complicated urinary tract infections and other serious infections caused by susceptible organisms.

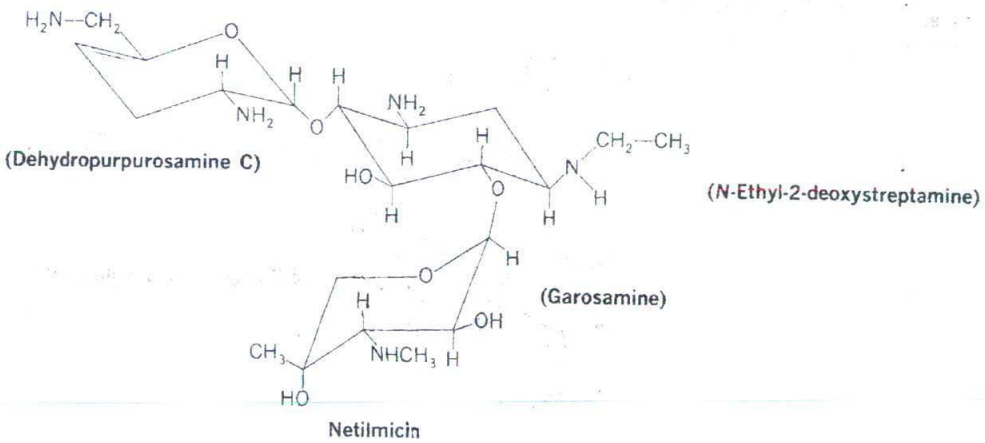
Netilmicin is available as the sulfate salt. The usual dosage regimen for patients with normal renal function is 4 to 6.5 mg per kg of lean body weight daily, intramuscularly or by intravenous infusion, in 2 or 3 divided doses for 7 to 14 days.

PRESCRIPTION PRODUCT. Netromycin®.

Spectinomycin

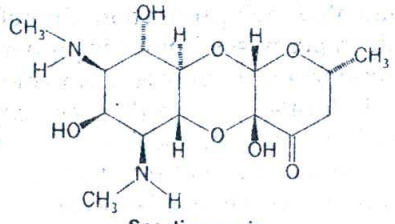
Spectinomycin is produced by *Streptomyces spectabilis* and *S. flavopersicus.* The antibiotic molecule is a glycoside, but it is not technically an aminoglycoside. An aminocyclitol aglycone is glycosidically linked to a neutral deoxysugar. The dry antibiotic powder is stable for long periods of time.

A number of the biologic properties of spectinomycin resemble those of the aminoglycoside antibiotics. It is not absorbed on oral administration, is excreted after in-



Netilmicin

jection in an active form by glomerular filtration, and acts by inhibiting protein synthesis through a mechanism involving the 30S subunit of the 70S ribosomal system. Spectinomycin has a broad antibacterial spectrum, but its only clinical indication is treatment of gonorrhea. Susceptible strains of *Neisseria gonorrhoeae* (MIC range of 7.5 to 20 μg per ml) are frequently controlled by a single parenteral dose of this antibiotic, a feature that is unusually advantageous for treatment of a venereal disease. This obviates many of the problems related to social stigma and mobile patient populations. Resistance to spectinomycin is known, and concern about facilitating the emergence of more resistance prompts some authorities to favor restricting the use of spectinomycin to cases in which penicillin is ineffective or contraindicated. Cross-resistance between penicillin and spectinomycin is unknown.



Spectinomycin

Spectinomycin is available as the pentahydrate of the dihydrochloride salt. The usual dose is 2 to 4 g intramuscularly; the higher dose is routinely recommended for female patients. Peak serum concentrations of 100 to 160 μg per ml occur in approximately 1 hour, 8-hour serum levels are 15 to 30 μg per ml, and total elimination of the antibiotic normally occurs within 48 hours. The most frequently observed adverse response is pain at the site of injection; dividing the dose between 2 sites, especially with higher doses of the antibiotic, has reduced this problem. Nephro- and ototoxicities have not been reported; this may be an inherent property of spectinomycin or it may reflect the short duration of the normal therapeutic regimen.

PRESCRIPTION PRODUCT. Trobicin®.

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